Animal and Cell Culture Models of PPGLs – Achievements and Limitations

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ABSTRACT

Research on rare tumors heavily relies on suitable models for basic and translational research. Paragangliomas (PPGL) are rare neuroendocrine tumors (NET), developing from adrenal (pheochromocytoma, PCC) or extra-adrenal (PGL) chromaffin cells, with an annual incidence of 2–8 cases per million. While most PPGL cases exhibit slow growth and are primarily treated with surgery, limited systemic treatment options are available for unresectable or metastatic tumors. Scarcity of appropriate models has hindered PPGL research, preventing the translation of omics knowledge into drug and therapy development. Human PPGL cell lines are not available, and few animal models accurately replicate the disease's genetic and phenotypic characteristics. This review provides an overview of laboratory models for PPGLs, spanning cellular, tissue, organ, and organism levels. We discuss their features, advantages, and potential contributions to diagnostics and therapeutics. Interestingly, it appears that in the PPGL field, disease models already successfully implemented in other cancers have not been fully explored.

Introduction

Paragangliomas (PPGL) are rare neuroendocrine tumors (NET), developing from adrenal (pheochromocytoma, PCC) or extra-adrenal (PGL) chromaffin cells, with an annual incidence of 2–8 cases per million. While most PPGL cases exhibit slow growth and are primarily treated with surgery, limited systemic treatment options are available for unresectable or metastatic tumors. While when detected early, PPGLs generally associate to a favorable prognosis, the nonspecific symptoms often lead to delayed diagnosis and initial presentation already with metastasis. In cases where surgical resection is not feasible, the available treatment options are limited and may only offer palliative relief, as there are currently no effective strategies for curing the disease in advanced stages.

PPGL exhibit the highest prevalence of genetically determined driver gene mutations among all human neoplasms, accounting for up to 40 % of all cases. The catalogue of germline pathogenic variants continues to expand and currently encompasses at least 20 susceptibility genes. Additionally, somatic mutations have been identified in another 30 % of cases, leaving approximately 30 % of PPGL cases with an unknown etiology (for a recent review of PPGL genetics refer to Cascón et al. [1])

Based on their transcriptome profile, PPGL tumors can be categorized into several clusters. The pseudohypoxic cluster, historically referred to as cluster 1, primarily contains most of the aggressive and metastatic cases. It is characterized by driver mutations affecting the TCA-cycle, particularly the succinate dehydrogenase complex, as well as mutations disrupting oxygen-sensing pathways, ultimately leading to activation of the hypoxia-inducible factor 2 alpha (HIF2α). Kinase-signaling, also known as cluster 2, encompasses pathogenic variations in genes such as *RET* and *NF1*, which activate tyrosine kinase pathways and confer constitutive proliferation signals. The identification of mutations impacting the Wnt pathway led to the recognition of the third main PPGL cluster caused by MAML3 fusions [2].

Distinct biochemical profiles and varying rates of metastasis have been associated with different clusters or subclusters. This heterogeneity extends to causes, symptoms, heritability, blood hormone levels, and metastatic potential within these tumor subsets. Effectively modelling each specific disease scenario would require tailored systems that account for this variability. However, laboratory models for studying PPGLs in general are quite limited, and specific prototypes designed to address driver mutations are missing.

To be relevant, a PPGL model not only should mirror the heterogeneity of the tumor cell features, but also recapitulate the tumor microenvironment (TME). The TME has garnered significant attention in current anti-cancer strategies, acknowledging that the complex composition of tissues, including tumor cells, endothelial cells, and immune cells, must be considered. In normal adrenal medullary (NAM) tissue, the primary cell types are the norepinephrine (NE) or epinephrine (EPI)-secreting chromaffin cells, surrounded by sustentacular cells. The extracellular matrix of chromaffin cells primarily consists of collagen type I, concentrated around each chromaffin cell, while collagen type IV and laminin encompass the Zellballen nests [3]. Alongside neural-crest derived cells, fibroblasts and a naturally high proportion of endothelial cells populate the tissue. The numbers of CD4 and CD8 immune cells are relatively low, whereas macrophages seem to play a more significant role [4, 5]. A comprehensive review of the PPGL microenvironment can be found in reference [6].

During tumorigenesis, the TME of PPGL tissue undergoes dramatic reorganization, depending on the cluster the tumors belong to. A recent study by Calsina et al. indicated substantial variability in immune-infiltrating cells based on the cluster and driver mutation. Traditional models for studying microenvironmental changes rely on animal models, particularly those with endogenous tumors. However, besides animal models, there may be additional options to capture the intricate interactions between tumor cells and the TME [7].

Another difficulty to faithfully model human PPGL is their typically very slow replication rate. In a cohort of 36 PPGL with VHL-driver mutations, tumors of 1–2 cm size grew with a rate of 0.03–0.32 cm/year [8]. In a cohort comprising 56 patients showing SDHx-affected PPGL, the average tumor doubling time was around 7 years [9]. However, clinical outcomes of metastatic PPGLs can be very variable, and they can also evolve in rapidly progressing tumors with high growth rates [10]. Therefore, probably more than one model is necessary to reflect the slow initial growth rate observed in most of the cases, and the rapid progression upon dissemination, as seen in some patients.

Chromaffin cells develop from multipotent Schwann-cell-precursors (SCP), emigrating from the neural tube into the developing adrenal [11]. During the maturation of terminally differentiated chromaffin cells, SCPs transit through a “bridge” and early connecting progenitor stage to early and late chromaffin cells. Single cell RNA-seq experiments from Zethoven et al. (2022 [5]) and Brazda et al. (2022 [12]) identified pseudohypoxic and, especially SDHx-associated PPGLs, to possess a less mature differentiation state, consisting mainly of early chromaffin cells with small proportions of connecting progenitor and bridge cells. In contrast, PPGL of the kinase-signaling cluster typically exhibit a mature, late chromaffin cell state [12]. Therefore, the cell differentiation stage might represent an additional relevant aspect to consider when modeling a specific PPGL tumor cluster.

In this review, we will discuss the currently available and potential models for PPGL research in the order of complexity ranging from cells to organisms (▶ **Fig. 1**).

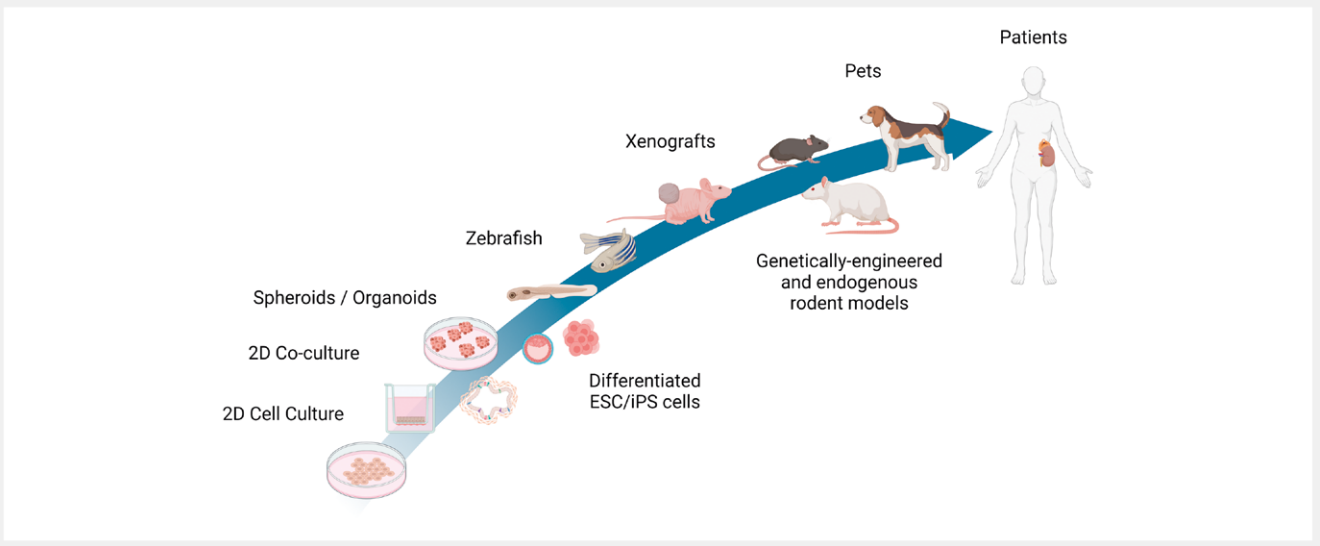
In vitro models

Working with primary PPGL cells *in vitro* poses significant challenges. Adrenal glands undergo autolysis shortly after death, likely due to the presence of abundant lytic enzymes [13]. Consequently, obtaining and processing primary tissue samples promptly is crucial, limiting the availability of human tissue for research purposes. Furthermore, even within tumors, chromaffin cells exhibit a slow replication rate, and the optimal composition and conditions necessary for their successful cultivation have yet to be fully determined. While a few historical reports mentioned the establishment of human PPGL cell lines, only a subset of these lines were adequately characterized, and unfortunately, they were never shared with the scientific community and may have been lost over time.

Tumor-derived cell lines are the standard *in vitro* cancer models. While it is relatively straightforward to obtain stable cell lines from some tumor entities such as, for example, breast cancer, resulting in over 80 different cell lines, the maintenance of proliferating NET cells in a differentiated state presents significant challenges [14].

Indeed, the numerous attempts to culture PPGL cells generated exceedingly few cell lines, suggesting that a conceptual step in the experimental protocol is missing. As previously noted, primary tumor tissues (derived from patients, animals, or xenograft models) contain several cell types in addition to chromaffin cells. Notably, tumor-associated fibroblasts exhibit accelerated growth rates compared to chromaffin cells, that could potentially outgrow the tumor cells themselves. Consequently, alongside confirming chromaffin cell identity using appropriate markers (e. g., CgA, Th, Syp, etc.), a simultaneous monitoring of their proliferation should be performed – a task easily achievable through methods like BrdU/EdU labeling. Conducting bulk analysis could otherwise potentially result in incorrect assessments of both the differentiation status and growth dynamics of chromaffin cells, which have demonstrated a remarkable ability to remain in a quiescent state for extended periods in culture [15].

Interestingly, the ongoing trials and failure to establish PPGL lines are often not reported in the literature but remain a recurring topic of discussion during informal meetings. Consequently, present-day PPGL cell line-based research predominantly relies on a limited number of well-characterized rodent cell models (▶ **Table 1**).



▶ **Fig. 1** Models of PPGL research (Created with BioRender.com) [rerif].

Rodent cell lines

PC12

The first PPGL cell line to be established was derived from rat PCC and was named PC12. It was developed by Greene and Tischler in 1976 [16] and remains the only publicly available *in vitro* PPGLs model [17]. These cells are valued not only for their ability to serve as chromaffin cell models capable of producing and secreting catecholamines but also for their potential to transdifferentiate into neuronal cells.

The PC12 cell line originated from an irradiated NEDH (New England Deaconess Hospital) rat predisposed to develop spontaneous PCCs, which were then transplanted into other rats [18]. After repeated *in vivo* passaging, stably proliferating cells (i. e., the PC12 line) could be obtained. It has been reported that culture conditions affect morphology and transcriptome of PC12 [19, 20]. Subsequently, it was discovered that PC12 cells harbor a chromosomal rearrangement in the *Max* gene (MYC-associated factor X), resulting in the loss of its protein function [20].

It took 17 years to recognize that pathogenic variants in this tumor susceptibility gene also cause hereditary and sporadic bilateral PCC with a relatively high risk of metastasis [21]. While *MAX*-mutated human tumors are assigned to the kinase-signaling cluster [22], it is interesting to note that PC12 cells also express HIF2α [23], a characteristic feature of the pseudohypoxic tumors. PC12 cells have maintained their characteristic features under appropriate culture conditions for over 40 years. However, a wide variety of morphologically and transcriptionally divergent variants have been reported [24] making the control of marker expression an essential task in experiments involving PC12 cells for correct data interpretation. As the original publication of PC12 establishment has been cited more than 7000 times, we refrain from summarizing the studies using PC12 cells appreciating that these cells are an important and crucial resource for more than the PPGL research.

MPC/MTT

The Mouse Pheochromocytoma Cells (MPC) encompass a collection of six cell lines derived from PCCs arising in irradiated heterozygous *Nf1*$^{+/-}$ knockout mice. Out of 44 mouse tumor preparations, four cell lines were obtained through primary cultures propagation, while the remaining two derived from subcutaneously transplanted tumors in mice (allografts). Among these lines, MPC 4/30/PRR is the most utilized one (often abbreviated only to MPC). The MTT (Mouse Tumor Tissue) cell line is a derivative of MPC 4/30/PRR (discussed further below) [25].

Inherited mutations in *NF1* are responsible for neurofibromatosis, a condition in which 0.1–5.7 % of patients develop pheochromocytoma [26]. Additionally, *NF1* pathogenic variants account for 41 % of sporadic cases of human PCC [21]. Currently, there is no study comparing the transcriptome of MPC cells to human PCC with *NF1* mutations using next-generation sequencing data available. However, previous studies have compared MPC cells to mouse NAM cells and observed overexpression of genes associated with neural progenitor lineages in the former [27]. *NF1*-mutated tumors belong to the kinase-signaling cluster, characterized by a more differentiated phenotype and epinephrine secretion. Four out of the six MPC cell lines maintained the expression of Phenylethanolamine N-Methyltransferase (PNMT), and electron microscopy revealed the presence of catecholamine-containing vesicles. However, the level of epinephrine secretion appears to be relatively low, despite the expression of the converting enzyme. Unlike PC12 cells, MPC cells do not differentiate into neurons upon nerve growth factor (NGF) stimulation due to the lack of TrkA receptor expression. However, they can differentiate in response to glial cell line-derived neurotrophic factor (GDNF) [28]. Furthermore, their high expression of RET makes them particularly interesting for drug studies targeting the kinase signaling pathways [29, 30].

The MTT line was obtained through repeated injection of MPC 4/30/PRR cells into the tail vein of mice [31]. MTT cells exhibit the ability to form solid liver metastases in nude mice and display a

▶ **Table 1** Cell lines models of pheochromocytoma and paraganglioma.

Cell line	Species	Catecholamine profile	Mutation	Chromaffin Markers	Morphology	Medium	Oxygen level	Repository/ Availability	Reference
MPC 4/30PRR	Mouse	DA, NE	*Nf1−/−*	PNMT, TH	Custers attached on collagen, neurite outgrowth	RPMI, 10% HS, 5% FBS	21 % O_2	From authors upon request	Powers et al. 2000 [23]
MTT	Mouse	DA, NE	*Nf1−/−*	PNMT, TH	Adherent, tend to form spheroid cluster spontaneously	RPMI, 10% HS, 5% FBS	21 % O_2	From authors upon request	Martinova et al. 2009 [31], Martinelli et al. 2022 [62]
imCC	Mouse	Nonfunctional	*SDHB−/−*	n. a.	Adherent (without coating), tend to cluster	DMEM, 10% FBS/4.5%	21 % O_2	From authors upon request	Letouzé et al. 2013 [45], Loriot et al. 2015 [48]
PC12	Rat	NE, DA, no EPI	*Max*	Low CgA, Syn, TH no PNMT	Spheroid cluster/round attached on collagen-coated tissue	RPMI, 10% HS, 5% FBS	21 % O_2	ATCC	Greene et al. 1976 [16]
RS0	Rat	DA, NE, no EPI	*SDHB−/− + (CDKN2A −/− CDKN2B −/−)*	CgA, TH	Spheroid cluster	RPMI, 2% B27, (serum free or 1% HS + 0.5% FBS possible)	5 % O_2	From authors upon request	Powers et al. 2020 [23]
RS1/2	Rat	DA, NE, EPI	*SDHB +/−*	CgA, TH	Spheroid cluster	RPMI, 2% B27, 1% HS, 0.5% FBS	5 % O_2	From authors upon request	Powers et al. 2020 [23]
hPheo	Human	Nonfunctional	*CDKN2A*	None (inducible with BMP4, NGF, Dexa)	Mesenchymal	DMEM/F12, 2% B27, 20 ng/ml EGF, 20 ng/ml bFGF2, ITS or ACL4	21 % O_2	From authors upon request	Ghayee et al. 2013 [50]
PTJ64p	Human	n. a.	*SDHC* c.43 C>T	n. a.	Adherent	DMEM-F12, 20% FBS	21 % O_2	Not available	Cama et al. 2013 [108], Florio et al. 2017 [109]
KAT45	Human	DA, NE, EPI	n. a.	n. a.	Singular, round	RPMI 1640, 10% FBS	21 % O_2	Not available	Venihaki et al. 1998 [43]
KNA	Human	Not detectable	*RET*	CgA, NSE, NE granules	Spheroid cluster	HAM F12, 10% FBS	21 % O_2	Not available	Pfragner et al. 1998 [42]
EPG1	Human	n. a.	n. a.	n. a.	?	EMEM, 10% FCS, NEAA, Mito + Serum Extender	21 % O_2	?	Stuschke et al. 1995 [44]

n. a.: Not available.

less-differentiated and more malignant phenotype compared to the parental MPC cells. Although injection of MTT cells has been established as a relevant model for malignant PPGLs, it should be noted that there are important differences between this model and metastatic human tumors [17], the most relevant being that the majority of the metastatic cases belong to the pseudohypoxic cluster.

Several modified MPC/MTT cell lines have been developed, which express marker proteins such as Luc and mCherry for imaging purposes [32]. Additionally, these cell lines have been engineered to insert or delete PPGL-driver genes, such as *Sdhb*, enabling the investigation of molecular changes caused by variations in the expression of such genes [33]. MPC/MTT lines were previously used for imaging studies [34, 35], drug testing [36, 37], and basic research [38, 39]. When conducting preclinical drug testing using these engineered cells it is important to remember that they have an underlying *Nf1* deletion (cluster 2) and therefore they have a mixed genetic background (Sdhb deficiency belongs to cluster 1), which may limit the translational value of the findings. A recent article by Mellid et al. (2023 [40]) highlighted the co-occurrence of *Nf1* pathogenic variants with other susceptibility genes such as DLST, MDH2, H3–3A, PRKAR1A, and ATRX, particularly for these novel gene combinations. The MPC/MTT cell lines will be good models to introduce these novel mutations to study transcriptional changes and assess drug vulnerabilities.

RS0 and RS1/2

In 2020, Powers et al. successfully generated the first heterozygous *Sdhb* knockout rat, which, following irradiation, developed PCCs and PGLs, albeit at a low frequency. Notably, the authors were able to generate mouse xenografts from primary rat tumors. RS0 mimics *SDHB*-deficient human paragangliomas, therefore, being the closest *in vitro* model to human *SDHB*-mutated PGLs currently available [23].

Genetically RS0 cells contain the deletions introduced by TALENs as in the parental rat cell. RS1/2 lost the mutated but maintained one WT-allele, making them heterozygous for *Sdhb*. This is consistent with the absence of SDHB protein in RS0 cells, while RS1/2 cell lines maintain its expression. Both lines harbor syntenic chromosomal deletions typically found in human PPGLs [23]. RS0 cells contain additionally homozygous deletions for *Cdkn2a* and *Cdkn2b*. The transcriptome profile of RS0 cells clearly demonstrates a pseudohypoxic signature, characterized by activation of HIF2α and its downstream targets. Consequently, RS0 clustered with the pseudohypoxic tumors in the human TCGA PPGL database. However, similar clustering was observed for RS1/2 cells and rat NAM, which only express minimal amounts of HIF2α. The authors initially suggested RS1/2 cells as control cells for RS0, but we believe these cell lines hold great potential as background model for the insertion of mutations/variants in other PPGL susceptibility genes, given their “close” resemblance to normal chromaffin cells. Notably, the generation of RS0 and RS1/2 cells involved the use of new culture conditions adapted from neurosphere cultures. For their maintenance, these cell lines require 5 % oxygen (O_2) levels. Unfortunately, not all laboratories have access to incubators with adjustable oxygen conditions. However, this issue can be addressed by utilizing small chambers that can be placed inside the incubators. Many tumor cell lines exhibit robust growth when exposed to O_2 levels of 21 %, commonly referred to as "normoxia." Regrettably, the detrimental impact of the ensuing stress response (as discussed in the review: Jagannathan et al. 2016 [41]) has been disregarded in numerous *in vitro* studies. It is worth noting that the physiologically appropriate O_2 levels, in healthy tissues, typically range between 1–9 % O_2. The heightened sensitivity of RS0 cells to oxygen may serve as a reminder that experiments involving chromaffin cells – natural oxygen sensors – should be conducted under appropriate conditions.

Human cell lines

(The lost) human PPGL lines

In 1998, two independent patient-derived PPGL cell lines were reported. Pfragner et al. presented the first human PCC cell line (named KNA) from a sporadic tumor carrying a RET C611Y-mutation [42]. This cell line would have been highly valuable as it not only represented a mutation commonly found in PPGLs but also exhibited positive staining for chromaffin markers such as human neurofilament, S100, NSE, somatostatin, GRP, VIP, dopamine β-hydroxylase, and substance P. The line was forming neurites in response to NGF. Catecholamine-secretion was not detectable, although dense vacuoles were visible in electron microscopy. From 12 injected nu/nu mice no PDX could be established and although the cell line was in culture for several years the cells finally lost their chromaffin identity and no earlier passage could be revived (Pfragner, personal communication) [42, 43]. The cell line, which was originally deposited in the European Collection of Authenticated Cell Cultures, has thus been retracted from the cell repository and is no longer available.

In the same year, Venihaki et al. [43] introduced KAT45 cells, a PPGL cell line derived from a 39-year-old female patient affected by Cushing’s disease. These cells produced NE and corticotropin-releasing hormone. Although morphologically similar to PC12 cells and responsive to NGF, the translational potential of these cells is limited due to the rarity of Cushing’s disease-associated PPGLs. Like the KNA cell line, also KAT45 cells were also lost over time [43].

A third human PPGL line, named EPG1 has been reported [44], but no characterization has been performed and it has not been used since.

Non-chromaffin cell lines

imCC

Several attempts have been made to establish immortalized cell lines from murine or rodent PCC models for drug testing and pathway analysis. One example of such cell models is the imCC cells, derived from primary *Sdhb* knockout mouse adrenomedullary cells (non tumor cells), as described by Letouzé et al. (2013) [45]. These cells have been shown to yield comparable results to primary PPGLs in various studies. For instance, they have investigated the role of PI3Ka inhibitor in PCCs [46], the metabolic plasticity of pseudohypoxic tumors [47], the involvement of hypermethylation and EMT in *Sdhb*-deficient cells [48], and the efficacy of combination therapies targeting these pathways [49]. However, in 2015, Loriot et al. [48] demonstrated that imCC cells exhibit mesenchymal characteristics, which are not typically observed in differentiated chromaffin cells. Therefore, while imCC cells may be useful for studying general effects of *SDHB* deletion, they may not be suitable for assays specifically related to chromaffin cell identity [45].

hPheo

The only currently available cell line derived from a human PPGL is hPheo1, described by Ghayee et al. in 2013 [50] as a PCC precursor cell line expressing a few neuronal and endocrine markers, which was subsequently immortalized with hTERT insertion. The cells were isolated from a human tumor, but are not reflecting terminally differentiated chromaffin cells, as hPheo1 do not contain the patients' pathogenic variations. Like RS0 cells, also hPheo1 cells acquired a *CDKN2A* mutation during subculturing, which was not present in the original patient's tumor. hPheo1 cells can be induced to express PNMT by using BMP4, NGF, and dexamethasone, which suggests their potential utility in studying differentiation processes. It would be interesting to explore the extent to which hPheo1 cells resemble SCP, which are the progenitors of chromaffin cells [51].

iPSC – differentiated chromaffin cells

In 2018, Abu-Bonsrah [52] demonstrated the generation of catecholamine-secreting adrenal chromaffin-like cells from human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) through a regime of BMP2 + FGF2 followed by a mixture of FGF2/dexamethasone/BMP4 and phorbol 12-myristate 13-acetate. The study demonstrated the expression of several relevant chromaffin markers and the secretion of NE and EPI by the differentiated cells. For how long these cells can be maintained in culture, and what is the potential for further expansion of the differentiated cells were not mentioned in the study. However, the derivation of iPSCs from patients, particularly those with germline pathogenic variants or variants of unknown significance, could serve as a valuable tool to study tumor initiation and development [53]. Furthermore, patient-derived iPSCs could be used for drug screenings in order to achieve a genetically-defined personalized therapy.

In theory, iPSCs can be also derived from cancer cells, including those with genetic rearrangements. However, while the creation of iPSC from blood cells is routinely done, establishing them from cancer cells is currently extremely challenging [54]. To the best of our knowledge, any iPSC approach has not yet been applied in PPGL research.

Co-culture systems

To simulate the tumor microenvironment and investigate the interaction between different tissue types, co-culture experiments can be employed. There are two common approaches: (1) using cell culture inserts having a membrane that allows only secreted factors to pass, or (2) direct cell-to-cell contacts of mixed populations. It should be noted that using mixed cell populations for co-cultures is typically limited to short-term experiments, as one cell type may outgrow the other over time.

In the context of *Sdhb*-knockout (KO) cells, Richter et al. speculated that the reduced growth observed *in vitro* may be due to a lack of support from the TME, as these corresponding human SDHB tumor cells grow aggressively *in vivo*. To test this hypothesis, they co-cultured primary fibroblasts with MTT-SDHB-KO cells and observed that the tumor cells proliferated at a faster rate in co-culture. This enhanced proliferation was in part attributed to the pro-mitogenic effect of lactate secreted by the fibroblasts [6].

PC12 cells have been co-cultured with various cell types in different studies. Interestingly, when co-cultured with endothelial cells, PC12 cells exhibited a stronger chromaffin cell phenotype and lost the capacity to form neurites in response to NGF stimulation [55].

Primary human cells

Primary 2D cultures

Due to the limited viability of primary PPGL cells in culture, there have been only few studies utilizing 2D cell cultures of human PPGLs. One study conducted by Santana et al. [56] employed a protocol typically used to generate neurospheres from the brain for the isolation of NAM chromaffin cells. The authors used DMEM/F12 medium supplemented with 1 % B27, EGF, and FGF2, which resulted in the formation of chromospheres – free-floating clusters of cells expressing progenitor cell markers such as Nestin, Ascl1, Sox9, Sox10, and Phox2b. However, they did not test for markers of chromaffin or neuroendocrine identity, such as dopamine-β-hydroxylase (DBH), synaptophysin (SYP), or chromogranin A (CGA). These chromaffin-like cells were able to differentiate into neurons when grown on coated wells with laminin and poly-l-lysine, and EGF was omitted while increasing the concentration of FGF2. The authors suggested that additional growth factors might be necessary to maintain proliferation specifically in a chromaffin cell population.

In the laboratory of Nölting, several studies have been performed using primary human pheochromocytoma cells to test the efficacy of drugs [46, 57]. However, the authors acknowledged that only short-term experiments were feasible to avoid fibroblast overgrowth in the culture.

Insights into the challenges of establishing cell lines from primary human PPGLs were recently reported by Bayley et al. [58]. The authors tested five different media compositions and found that a serum-free neuroblastoma medium to be the most suitable. Additionally, they demonstrated that lactate had a promoting effect on cell proliferation and long-term survival.

Primary 3D organotypic cultures

A major limitation in cancer research is the low translation rate of preclinical drug testing to clinical outcomes. As a result, various alternative *in vitro* models have been developed to better replicate the complexity of the tumors thereby increasing their predictive value. These models include organoids, cell-aggregate cultures, spheres, tissue explants, tissue slices, and organs-on-a-chip.

Studies have revealed that tumor cells selected for rapid *in vitro* growth undergo changes in the expression of cell cycle genes and metabolic processes, leading to a gradual divergence from the original tumor [59]. Several studies showed that growth of tumors in 3D cultures are more suitable to maintain original cell characteristics. Freshly isolated normal chromaffin cells and PPGL cells form floating clusters referred to as chromo- or neurospheres. These cell aggregates represent the simplest form of 3D *in vitro* culture. However, the precise composition of these floating clusters has not been extensively studied and characterized.

Cells, able to adhere to the culture dish, are typically suitable for spheroid cultures. Various techniques for spheroid formation exist, including hanging drops and ultra-low attachment plates. Our own unpublished data suggests that isolated primary rat PCCs and PC12 cells do not form tight spheres naturally, but the addition of extracellular matrix components can enhance their formation. In contrast, spheroids of MPC and MTT cells have been successfully generated and used for drug and therapy studies [46, 60–62].

Additionally, primary human PPGL 3D spheroids have been obtained by growing cells in ultra-low attachment plates immediate-

ly after isolation, enabling their utilization for drug screening [57]. However, a comparison of the efficacy of 2D versus 3D culture was not performed, likely due to the limited availability of primary tumor tissue.

Dahia's presentation at the ISP meeting in 2022 highlighted promising results in the culture of PPGL organoids using Matrigel rings. Embedding the organoids within Matrigel rings simplifies the process of exchanging the medium in the wells, enabling high-throughput drug screening [63]. This approach holds potential for advancing drug discovery and screening in the field of PPGL research. To our knowledge, the experiments are so far limited to short-term treatments, as continuous organoid cultures have not been reported for PPGLs yet.

Organotrophic adrenal tissue slices (TS) have been used since several years to study changes in catecholamine-secretion upon electrophysiological stimulation [64–66]. TS cultures maintain the original TME, including various cell types and their crosstalk, thus providing a more physiologically relevant model. They have been employed in drug response and toxicity studies for various tumor types but not for PPGL, yet. However, there are some limitations associated with this technique, such as the technical challenges in preparing standardized slices for consistent treatment outcomes and the relatively short duration the tissue can be maintained in culture. TS cultures are relatively cost-effective and demonstrate high predictability of treatment outcomes in other tumor types. Given its potential, it would be interesting to further explore the applicability of this model to PPGL research.

Organism-based model systems

While the advancements in 3D cell culture techniques are giving new hope that the gap between *in vitro* research findings and clinical translation can be narrowed, some aspects of cancer research are still limited to animal studies. Among them are tumor initiation and progression, which can be modelled at best in a complete organism. Interestingly, some PPGL susceptibility genes were first identified in animal models before their relevance in human tumors became known, be it because of the rarity of the disease or partial penetrance due to uncommon inheritance patterns.

Therefore, to gain a deeper understanding of tumor heterogeneity, tumor progression and metastasis, as well as host-tumor interactions, and the role of the TME, it is crucial to develop models that recapitulate the situation in patients and are translatable to clinical settings. Such models could also be exploited for early detection of PPGLs, and for drug screening. While simple and cost-effective model systems such as yeast, worms, and fruit flies have proven useful for studying basic gene function, they lack the complexity required to mimic NETs in their natural environment. Insects and nematodes do not possess adrenals, therefore are not suitable to study tumorigenesis. However, deletion of *Sdhb* in C. elegans and Drosophila was associated to hypersensitivity to oxygen (reviewed in [67] by Takács-Vellai et al., 2021).

Zebrafish

Zebrafish have emerged as a promising model organism for cancer research due to several key characteristics. Firstly, their transparent embryos enable direct visualization of tumor development and progression in real-time, providing invaluable insights into the underlying mechanisms of cancer growth. Secondly, zebrafish exhibit a high degree of genetic and physiological similarity to humans, despite lacking an adrenal gland. Instead, they possess an analogous organ called the interrenal gland, which houses chromaffin cells [68]. Additionally, studying therapeutic interventions in zebrafish larvae is advantageous as compounds can be added directly to their swimming water, simplifying drug screening.

Zebrafish *Vhl*-knock out (KO) mutants have been successfully generated, but unfortunately, the larvae exhibit a significantly reduced lifespan, typically dying within 9–10 days of development. Like mammalian chromaffin cells, the deletion of *Vhl* in zebrafish leads to the activation of the HIF signaling pathway, resulting in increased production and secretion of normetanephrine. Consequently, these larvae showed elevated heart rate and blood pressure. In a study utilizing this model, researchers investigated the interplay between HIF signaling, glucocorticoid transcriptional activity, and cortisol biosynthesis [69].

In 2021, Dona et al. published a *Sdhb*-KO model in zebrafish which led to loss of complex II activity and elevated succinate levels mimicking the features of *SDHB*-associated PPGLs in humans [70]. The researchers explored the impact of ascorbic acid on the redox balance of the *Sdhb*-KO zebrafish larvae, suggesting that the model is a potentially valuable tool to screen for drugs effective against SDHB-associated pseudohypoxic PPGLs [71]. This model opens new possibilities to understand the molecular mechanisms associated to *SDHB* deficiency, and to investigate novel therapeutic interventions for *SDHB*-related tumors.

Mice

Genetically modified mouse models

Pseudohypoxia cluster Over the past three decades, several genetically engineered (▶ **Table 1**) mouse models have been generated to explore the mechanisms and conduct pre-clinical studies on PPGLs. However, only PCCs related to Cluster 2 have been successfully modelled in mice thus far, whereas no tumors belonging to the pseudohypoxc cluster of PPGLs have ever developed in the various animal models of defects in *SDHB*, *SDHD*, *FH*, *EPAS1*, or *VHL*. Studies [72, 73] have demonstrated that homozygous inactivation of *Sdhd* resulted in embryonic lethality. Similar outcomes were observed with *Sdhb*$^{+/-}$ mice generated [74], while complete knockout of *Sdhb* led to embryonic lethality, mirroring the results seen with *Sdhd*, *Fh*, and *Vhl* [47]. The disappointments in developing a model predisposed to the formation of pseudohypoxic tumors were disheartening until recently (excellent review in [75] by Lepoutre-Lussey et al., 2016). Armstrong et al. (2022) made a breakthrough by combining the DESI-MSI and Cre reporter system to create a mouse model with a non-lethal *Sdhb*-deficient adrenal medulla. While the chromaffin cells exhibited elevated succinate levels, the induction of tumorigenesis required an additional loss of *Nf1*. Although concurrent biallelic *SDHB* and *NF1* mutations have not been observed in humans, both genes are implicated in PPGL and some common tumorigenic mechanisms have been identified (Armstrong et al., 2022 [76]). A similar double conditional knockout was performed by Lussey-Lepoutre- et al. (2015) [47] in Pten$^{+/-}$ mice with heterozygous *Sdhb*, resulting in PCCs with a comparable incidence to Pten-deficient mice alone.

Models that aimed at reproducing the pseudohypoxia cluster with activated HIF2α signaling did not result in PPGL development either. Wang et al. presented a mouse with a gain-of-function mutation in *EPAS1* [77]. The mouse displayed elevated levels of erythropoietin and polycythemia, a decreased urinary metanephrine-to-normetanephrine ratio, which could be partially reversed by the inhibitor PT-2385. Yet, the model did not develop any tumors. *Vhl*$^{-/-}$mice were also embryonic lethal and tissue-specific KO still did not result in tumor formation [78]. A *Phd2*-deficient mouse developed adrenals with gene expression signature of an HIF2α downstream target activation, and a NE-secretory phenotype. However, the chromaffin cells did not show any signs of malignant transformation besides the developmental induced mislocalization to regions outside of the cortex. It would be interesting to investigate, whether radiation could induce tumor formation in these mice.

Kinase-signaling cluster In contrast to the pseudohypoxic cluster, several animal models of PPGL belonging to the kinase-signaling cluster were reported. *Nf1*$^{+/-}$ mice develop PCC (see MPC line development above) [79]. Transcriptome analysis of these tumors revealed an increase in the expression of developmentally regulated genes, and the overexpression of the *RET* protooncogene [80]. The mice were used to study tumor formation in context of radiation and chemical induction [81]. RET pathogenic variants are associated with the multiple endocrine neoplasia type 2 (MEN2) syndrome, which includes PCC among the associated tumors. *Ret*$^{Met-918Thyr}$ MEN2 mice develop benign PCC at an age of 6–8 month with up to 100 % incidence [82]. Schulz et al. (1992) [83] described c-*mos* transgenic mice lines that present similar tumor patterns as MEN2 patients and develop bilateral PCCs with up to 58 % penetrance. While *mos* expression is not always correlated with the MEN2 syndrome in humans, these models yielded kinase-signaling-representative PCCs within 8 months of age and at a good frequency [84].

Interestingly, other mouse models with the overexpression/deletion of oncogenes or tumor-suppressors not typically involved in the human disease, also developed PPGL in mice (summarized in ▶ **Table 2**).

The role of background and radiation on tumor formation In the literature, two prominent factors, in addition to driver gene mutations, affect PPGL development in mice, that is, radiation and background strain. Low gamma ionizing radiation one week postnatally has been found to increase by 8-fold the rate of PPGL formation in mice (e. g., *Nf1*$^{+/-}$ mice) and to also promote the rate of PPGLs in rats [79].

▶ **Table 2** Mouse models of pheochromocytoma and paraganglioma.

Cluster	Mouse model		Penetrance	Age of dev. in months	Reference
Kinase-Signaling Cluster	Nf1	Nf1$^{+/n31}$	10 to 20 % PCC	18–24	Jacks et al. 1994 [79]
		Nf1$^{+/n31}$ irradiated	87 % PCC	12–15	Powers et al. 2000, [23] Tischler 1995 [95]
	Ret	Ret$^{Met918Thr/Met918Thr}$	100 % PCC	6–10	Smith-Hicks et al. 2000 [80]
	c-mos	LTR:c-mos	58 % PCC (bilateral)	8	Schulz et al. 1992 [83]
	p27Kip1	p27$^{Kip1-/-}$	24 % PCC	12	Franklin et al. 2000 [110]
		p27$^{Kip1-/-}$ p18$^{Ink4c-/-}$	91 % PCC	3	Franklin et al. 2000 [110]
		p27$^{Kip1+/-}$ p18$^{Ink4c-/-}$	17 % PCC	7	Franklin et al. 2000 [110]
		p27$^{Kip1-/-}$ p18$^{Ink4c+/-}$	50 % PCC	7	Franklin et al. 2000 [110]
		p27$^{Kip1-/-}$ Pten$^{+/-}$	100 % PCC	3–5	Di Cristofano et al. 2001 [111]
	Pten	Pten$^{+/-}$	100 % PCC	9–16	Di Cristofano et al. 2001 [111]
		Pten$^{+/-}$ Ink4a$^{Arf+/+}$	24 % PCC	10.5	You et al. 2002 [112]
		PSA-Cre Pten$^{f/f}$	100 % PCC	15–16	Korpershoek et al. 2009 [105]
		Pten$^{+/-}$ Ink4a$^{Arf+/-}$	57 % PCC	7.5	You et al. 2002 [112]
		Pten$^{+/-}$ Ink4a$^{Arf-/-}$	59 % PCC	6	You et al. 2002 [112]
	p18^{Ink4c}	p18$^{INK4c-/-}$	8 % PCC (4 % metastasis)	12	Franklin et al. 2000 [110]
	Rb	Rb$^{+/-}$	71 % (14 % bilateral) PCC	12	Nikitin et al. 1999 [113]
		Rb$^{-/-}$, p130$^{-/-}$	55 % PCC		Dannenberg et al. 2004 [114]
		Rb$^{+/-}$, p130$^{+/-}$	55 % PCC		Dannenberg et al. 2004 [114]
		Rb$^{F2/F2}$-Trp53$^{F2-10/F2-10}$	100 % PCC	4	Tonks et al. 2010 [115]
		Rb1$^{F2/F2}$, Trp53$^{F2-10/F2-10}$, TEC1	100 % PCC (bilateral)	4	Tonks et al. 2010 [115]
	ErbB-2	ErbB-2Δ-OE	5 % hypertrophy, 5 % PCC (only males)	15	Lai et al. 2007 [116]
	CerS2	CerS2$^{GT/GT}$ (*CerS2null*)	100 % medullary hyperplasia, 54 % PCC (bilateral)	13	Park et al. 2015 [117]

▶ **Table 2** Continued.

Cluster	Mouse model		Penetrance	Age of dev. in months	Reference
Pseudohypoxia Cluster	Epas1	Epas1$^{-/-}$	Lethal on congenic, F1 hybrid (129-Epas1 + / − × C57-Epas1 + / −), 0 %		Scortegagna et al. 2003 [118]; Tian et al. 1998 [119]
		Epas1$^{+/-}$	no		Peng et al. 2011 [120]
		Th-CreER Epas1$^{f/f}$	no		Macias et al. 2018 [121]; Fielding et al. 2018 [122]
		Epas1^{A529V}	no		Wang et al., 2019 [77]
	Vhl	Vhl$^{-/-}$	Embryonic lethal		Gnarra JR et al. 1997 [78]
		Vhl$^{+/-}$	no		Ma et al. 2003 [123]
		Th-Cre Vhl$^{f/f}$	no		Macias et al. 2014 [124]
	Phd2	Phd2$^{-/-}$	no		Eckhardt et al. 2021 [125]
		Th-Cre PhD2$^{f/f}$	no		Eckhardt et al. 2021 [125]
		Th-CreER PhD2$^{f/f}$	no		Fielding et al. 2018 [122]
	Sdhb	SDHB$^{-/-}$	no		Letouzé et al. 2013 [45]
		SDHB$^{+/-}$	no		Lepoutry-Loussey et al. 2016 [75]
		Th-Cre-SDHB$^{f/f}$	no		Lepoutre-Lussey et al. 2016 [75]
		PSA-Cre SDHB$^{f/+}$/PTEN$^{f/f-}$	42 %	10	Lepoutre-Lussey et al. 2016 [75]
		SDHB$^{+/-}$, PTEN$^{+/-}$	80 %		Lepoutre-Lussey et al. 2016 [75]
		Th-cre SDHB$^{f/f}$, Nf1$^{f/f}$	76 % PCC	12	Armstrong et al. 2022 [76]
	Sdhc	*Th-Cre-SDHC$^{f/f}$*	no		Al Khazal et al. 2020 [126]
		Th-Cre-SDHC$^{f/f}$LSLp53/+	no		Al Khazal et al. 2020 [126]
	Sdhd	−/−	Embryonic lethal		Piruat et al. 2004 [72]
		+/−	no		Bayley et al. 2009 [73]
		ESR-SDHD$^{+/-}$	no		Diaz-Castro et al. 2012 [127]
		Th-Cre SDHD$^{f/f}$	no		Diaz-Castro et al. 2012 [127]
	Fh	Fh1$^{-/-}$	Embryonic lethal		Pollard et al. 2007 [128]
		Ksp1.3-Cre-Fh1$^{f/f}$	no		Pollard et al. 2007 [128]

Outbreeding the 129SV-*Nf1*$^{+/-}$ mutation carriers to WT C57BL6 mice was found to be crucial to promote tumorigenesis, surpassing the effect of radiation exposure [23]. The effect of the genetic background on PPGL formation may stem from a strain-dependent expression of tumor suppressor genes [85]. Thus, it might be worth to backcross lines that develop PPGL at low frequency onto other strains to increase the rate of tumor development.

Xenograft and allograft models of engrafted cell lines

Xenograft models based on the engraftment of the above-mentioned PPGL cell lines, parental and genetically engineered, have been generated. Expression of marker proteins such as luciferase (MTT-luc) [86], or fluorescent proteins (MPC-mCherry) have been successfully used to monitor tumor growth and drug behavior using imaging (reviewed by Mohr et al., 2021) [87]. MTT-S*dhb*$^{-/-}$ cells were also used to allograft nude mice by Pang et al. (2017) to study the effects of chemotherapy [88]. PC12 cell xenografts were used to study the efficacy of mTORC1/2 or RTK inhibitors [89] and other targeted therapies or in studies related to metastasis formation [90]. Highly promising are RS0 xenografts, for which no studies besides the original article have been published so far but are very likely to come.

Patient-derived xenografts

Patient-derived xenograft (PDX) models involve engrafting freshly-isolated human tumor tissues/cells into immunodeficient mice. These models have been shown to preserve key features of the original tumors thereby offering a valuable platform for personalized treatment strategies, able to bridge the gap between preclinical and clinical studies. In 1999, Gross et al. successfully implanted 2–3 mm tumor pieces from a PGL with a VHL mutation into Balb/c nu/nu mice, resulting in PDX growth up to 1 cm. This model was used for therapy studies with Linomide [91].

In 2017, Powers et al. reported the successful engraftment of 3 out of 13 transplanted PGL samples into NSG mice, with visible PDX formation after 10 months [92]. Due to the slow proliferation of PPGL cells, the obtained xenografts were small and no further PDX

▶ **Table 3** Studies reporting generation of PDX from PPGL tissue.

Type of cells injected	Engraftment rate	Duration until tumor growth	Mode of transplantation	Mouse strain	Chromaffin markers in PDX	Reference
Primary PGL (VHL-Val166Phe)	4/20	7 months	subcutaneous, 2–3 mm pieces	Balbc nu/nu	CgA, NSE	Gross et al. 1999 [91]
Primary PGL (mainly SDHx)	3/13	10 months	subcutaneous, thawed cells	NSG	TH, morphology reflected original tumor	Powers et al. 2017 [92]
Primary HNPGL	80/90	4.5–10 months	subcutaneous, 3 × 3 mm pieces	NSG	no	Virginelli et al. 2018 [93]

passage could be obtained. As the engrafted sample material was derived from cryopreserved cells, the authors suggested that primary tumor tissues (with supporting tumor-associated cell types) and a greater cell number might be a more promising starting material. A year later, in a study by Verginelli et al., 90 tumor samples from 16 PGL patients were implanted as 3 × 3 mm pieces into NSG mice. A remarkable engraftment rate of 89 % was achieved. Interestingly, the PDXs exhibited mesenchymal-like progenitor characteristics and lacked the expression of CgA or SYP, indicating a change from the chromaffin cell identity or overgrowth of other cell types from the initial tumor material [93], different to the findings of Powers et al. and Gross, who reported the maintenance of parental tumor characteristics in their PDX model. All studies reported extended periods of several months until tumors grew in the PDX models (▶ **Table 3**). Despite the limitations and differences observed, the establishment of PDX models holds enormous potential for personalized medicine approaches. The development and characterization of PDX models, especially the establishment of continuously growing PDX lines, can provide valuable translational tools to study tumor biology and treatment response.

Rats

Spontaneous or chemically-induced

Spontaneous PPGLs, particularly PCCs, are common in rats, with studies reporting an incidence ranging from 17–30 %. The prevalence depends on rat strain, age, and gender, with males more frequently affected than females [94]. In toxicology studies rats have shown an elevated incidence of PPGL formation as a result of exposure to different substances. Although the relevance of these substances to humans remains uncertain, the fact that these substances are associated with hypoxia, oxidative phosphorylation uncoupling, metabolic impairment, disruption of calcium homeostasis, and hypothalamic endocrine axis disturbance [94] recapitulates the current understanding of the molecular mechanisms underlying human PPGL. Interestingly, studies by Tischler et al. showed that Vitamin D is the most powerful mitogenic stimulus for adrenal chromaffin cells in rats [95], but it has no effect on chromaffin cells proliferation *in vitro*. As mentioned above, irradiation is also a very effective method to induce PPGL in rats.

SDHB rats

The search for an animal model of *SDHB*-associated PPGL followed a long and hard path. Several attempts to model the disease in mice failed. Finally, Powers et al., were able to generate three rat lines in which small *Sdhb* deletions were inserted via TALENs [23]. The strain with a 13 bp deletion was further investigated. Embryonic development of $Sdh^{-/-}$ animals is stalled at day 5–6. However, heterozygous animals were viable and developed PCC and PGL. As the incidence of tumor formation was rather low (1 big, 4 micro PCCs in 13 animals), animals were irradiated with 5 Gy of gamma irradiation 1 week postnatally. In this group, the incidence of PCC increased to 3 big, 6 micro PCCs in 16 animals. In humans, 42 % of the *SDHB* mutation carriers develop PPGLs and up to 60 % of them metastasize [96]. The reason why in rats and mice the deletion of *Sdhb* does not associate to such an aggressive behavior is still unknown.

MENX

In 2002, a unique rat strain was discovered (named MENX) showing the predisposition to develop multiple endocrine neoplasias, including pituitary tumors NETs, pancreatic beta cell hyperplasia, parathyroid and thyroid hyperplasia, PCC, and PGL. The driver genetic alteration causing MENX is a frameshift mutation in the *Cdkn1b* gene, which encodes the cell cycle regulator p27kip1 (p27). This mutation causes a loss of p27 function in MENX rat cells. Subsequently, it was found that mutations in the human homolog *CDKN1B* cause a similar syndromic presentation of multiple endocrine tumors, named multiple endocrine neoplasia type 4 syndrome (MEN4) [97]. The MENX rat syndrome was initially thought to follow a recessive inheritance pattern. However, it was later determined that also heterozygous mutant rats have a tumor phenotype similar to the homozygous ones with tumor development being slower in the former. This is caused by p27 haploinsufficiency. Homozygous rats develop bilateral PCCs at 8–10 months of age with 100 % penetrance, as well as abdominal PGLs (frequency approx. 10 %), heterozygous rats at an age of 15–18 months. Tumor onset seems to be earlier in male *versus* female rats, and macroscopically detectable PGL are more prevalent in heterozygous animals at an older age. PCCs in MENX exhibit a pseudohypoxic signature, characterized by stabilized HIF2α and activation of its downstream targets [98]. The earliest detectable changes involve mitochondrial morphology at 1 month of age with loss of cristae and ballooning of the mitochondria. During the transition from hyperplasia to

PCC, various alterations in TCA-cycle metabolites occur, particularly the accumulation of 2-hydroxyglutarate. This metabolite is known to inhibit TET enzyme function, which correlates with a hypermethylated phenotype and loss of the 5-hmC mark. MENX PCCs exhibit a noradrenergic phenotype, with loss of PNMT and EPI secretion dependent on tumor size. Around 10 % of cases show large tumors that evade the capsule. Untreated animals with PCC experience high-blood pressure-induced end-organ disease in the heart and kidneys [99]. Altogether, this model recapitulates the most relevant features of human pseudohypoxic PPGL. The PPGL in MEN4 have not been documented to date, whereas bilateral PCC occur with complete penetrance in MENX rats. This is possibly due to the predisposition of rats to spontaneously develop PPGL. Nevertheless, MENX rats have played a crucial role in studying the molecular underpinnings of PPGL initiation and progression, in identifying novel therapeutic targets for these tumors, in evaluating novel functional imaging techniques, and to test clinically relevant therapies [98, 100–102].

Dogs

Although not as frequent as in rats, it has been reported that PPGLs are more prevalent, in dogs than in humans [103]. Tumors in dogs are aggressive and intra-adrenal. The clinical presentation in dogs (e. g., hypertension) is similar to that in humans and therefore suitable for imaging modalities used in patients like scintigraphy and computed tomography [103]. This makes dogs an interesting model to conduct treatment studies where tumor monitoring is done using imaging. Given the rarity of human PPGLs, spontaneous canine cases can be exploited for better understanding of the disease. This seems highly plausible as human and canine PPGLs share analogous genomic alterations and metastatic pathways, especially with tumours carrying *SDHx*-related mutations [104, 105]. Dogs are one of the few models where studies of metastatic PPGLs are possible. Recently, Enright et al. studied the effect of pre-operative treatment with alpha-blockers on short- and long-term survival in dogs with PCC [106] and found no association between alpha-blockers therapy for hypertensive dogs with PPGLs with post-operative survival. If performed on numerous dogs affected by PPGLs, such studies might have a translational potential to human cases on a topic (the utility of alpha-blockers) that still raises continuous discussion in the PPGL community.

Concluding remarks

Working on a rare disease without proper *in vitro* and *in vivo* models is challenging. Chromaffin cells do not grow with traditional cell culture methods, and improved media compositions are necessary to support their proliferation *in vitro*. The advancement of iPSC technology rises hope for alternative cell models. TS culture has not applied for PPGL research yet, but the conservation of TME is an advantage that makes it worth to try. Furthermore, we join the opinion raised by Bayley et al. [17] that it is important to share both successful and "negative" studies regarding the establishment of cell lines to learn from each others' experiences. Novel advances on PPGL models as presented at the ISP2022 meeting were recently summarized by Tischler and Favier [107]. With the availability of suitable models, conducting large-scale drug screening in zebrafish larvae can provide new therapeutic possibilities. While murine models have not yielded the desired results, exploring *Vhl1*, *Epas1*, and *Fh* mutations in rats may offer a more suitable approach given their biological characteristics that favor PPGL development.

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Conflict of Interest

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