



Pheochromocytoma/paraganglioma: recent updates in genetics, biochemistry, immunohistochemistry, metabolomics, imaging and therapeutic options

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Abstract: Pheochromocytomas and paragangliomas (PPGLs), rare chromaffin/neural crest cell tumors, are commonly benign in their clinical presentation. However, there are a number of cases presenting as metastatic and their diagnosis and management becomes a dilemma because of their rarity. PPGLs are constantly evolving entities in the field of endocrinology brought about by endless research and discoveries, especially in genetics. Throughout the years, our knowledge and perception of these tumors and their genetic background has greatly expanded and changed, and each new discovery leads to advancement in the diagnosis, treatment and follow-up of PPGLs. In this review, we discuss the recent updates in the genetics, biochemistry, immunohistochemistry, metabolomics, imaging and treatment options of PPGLs.

Keywords: Diagnosis; genetics; paraganglioma; pheochromocytoma; treatment

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Introduction

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors (NETs) producing catecholamines and originating from adrenal medulla chromaffin cells or from neural crest cells outside the adrenal gland. In 80% of cases, these tumors arise in the adrenal medulla and in the remaining 20%, tumors arise outside the adrenal glands in the prevertebral and paravertebral sympathetic ganglia located mainly in the chest abdomen and pelvis (1,2). Using the latest WHO definitions, the term pheochromocytoma is used for tumors arising from the adrenal gland while tumors which arise outside the adrenal gland are termed as paraganglioma (3). Increased levels of catecholamines accounts for characteristic clinical manifestations

particularly elevated blood pressure, headache, palpitations, and diaphoresis. PPGLs have an approximate incidence of 2–8 individuals per million population per year accounting for approximately 0.1% of individuals with hypertension (4). Most PPGLs are benign but there are a number of cases presenting initially as metastatic with an approximate incidence of one per million population per year. Patients with metastatic PPGL have a survival rate of 40–77% in five years and progression free survival ranges from 4–36 months (5). Metastatic PPGLs behave in a variable manner with some initially presenting with metastases and some developing metastases years after the initial diagnosis of PPGL. Factors correlated with an accelerated disease progression include male sex, diagnosis at an older

age, synchronous metastases, bigger tumor size, increased dopamine level and failure to remove the primary tumor (6).

Genetics

PPGLs have a high degree of heritability with 40% of cases carrying a germline mutation. Throughout the years, there has been more than 20 susceptibility genes identified. The underlying mutation influences PPGL clinical presentation such as cell differentiation, specific catecholamine production, tumor location, malignant potential and genetic anticipation (7-9) (Table 1). The Cancer Genome Atlas divided PPGL into 3 clinically useful molecularly defined groups: Kinase signaling subtype, Pseudohypoxia subtype, and Wnt-altered subtype (20).

Kinase signaling subtype

This subtype consists of somatic and germline mutations in *NF1*, *RET*, *TMEM127* and *HRAS* genes (Table 1). Patients with these mutations typically present with pheochromocytoma having adrenergic biochemical phenotype corresponding to its high expression of phenylethanolamine N-methyltransferase (PNMT) that converts norepinephrine to epinephrine (20). Most of these pheochromocytomas are benign but have high degree of recurrence and multiplicity.

Pseudohypoxia subtype

Patients in this subtype present with both pheochromocytoma and paraganglioma and may produce either norepinephrine or dopamine or both. Thus, typically, epinephrine and metanephrine levels are within normal limits. This subtype is further divided into two main subgroups: tricarboxylic acid cycle (TCA)-related PPGLs that include mainly succinate dehydrogenase subunits A-D (*SDHx*), fumarate hydratase (FH), isocitrate dehydrogenase (IDH), and *VHL/EPAS*-related mutations. *SDHx* mutations are associated with PPGLs as well as gastrointestinal stromal tumors, pituitary adenomas, chondromas, neuroblastomas and very rarely gastroenteropancreatic tumors (21-24). *SDHB* mutation in particular was found to increase the risk for clinically aggressive PPGLs that are more likely to develop metastases or locally aggressive or recurrent tumors (7,25,26). In fact, *SDHB* mutation greatly affects outcome among patients with metastatic PPGLs exhibiting less disease-free interval and a shorter time interval between

identification of the disease and first evidence of metastases (25,27). Interestingly, when compared to *SDHD* variant carriers who have a standard mortality ratio (SMR) of 0.93 which is comparable to the general population, *SDHB* variant carriers have a greater SMR at 1.89 which increases to 2.88 among *SDHB* variant carriers with a personal history of PPGL (28). Given its risk for metastases and association with poor outcomes, multiple studies have been done to determine the PPGL penetrance among individuals with an underlying *SDHB* mutation. A study showed that *SDHB* mutation has a PPGL penetrance of 49.80% at 85 years of age (29). Surprisingly, a difference in the age-related PPGL penetrance was noted between males and females with males having a 50% PPGL penetrance at age 74 but this was not reached in females (29). In addition, metastasis was noted in 85 out of 143 patients with PPGL (59.44%) with a median time of 3 years between initial diagnosis of PPGL and documentation of metastases. In another study, *SDHB* mutation was found to have a penetrance of 21% at 50 years of age but in contrast, there was no difference in the age-related PPGL penetrance between males and females (30). Benn *et al.* developed an approach to estimate lifetime disease penetrance of *SDHx* mutation by comparing allelic frequencies among individuals with and without PPGL (31). Using this approach, *SDHB* variants have an estimated lifetime disease penetrance of 22% as compared to *SDHC* and *SDHA* variants which have an estimated lifetime disease penetrance of 8.3% and 1.7% respectively.

FH mutation predisposes an individual to a syndrome of leiomyomatosis, renal cell carcinoma together with pheochromocytoma or paraganglioma (32-34). *FH*-related PPGLs are often metastatic or multiple. On the other hand, an *EPAS1* mutation, also known as *HIF2A* mutation, results in a syndrome of multiple PPGLs, duodenal somatostatinomas and polycythemia also known as the Pacak-Zhuang syndrome with a high metastatic potential and multiplicity (35-38).

Wnt-altered subtype

This consists of adrenal pheochromocytomas associated with *CSDE1* somatic mutations and *MAML3* fusion genes activating the Wnt and Hedgehog signaling pathways (20). There are no known germline mutations in this subtype making it specific for sporadic pheochromocytoma. In patients with these mutations pheochromocytomas present as recurrent or metastatic.

Crona *et al.* looked into PPGLs in a PanCancer perspective (39). The PanCancer Initiative aims to ascertain similarities across various types of cancer and cell origin or within groups found to be associated based on anatomical or morphological characteristics (40). In the analysis of Crona *et al.*, they found that PPGLs clustered with pancreatic NETs and neuroblastomas which challenges the current clinicopathological classification of PPGLs. PPGLs were found to share similarities with neuroblastomas, glioblastoma multiforme and brain lower grade glioma neuroblastoma. These similarities can be used to make careful conclusions about the developmental origin of these tumors or the role of microenvironment where these cancer cells develop from their precursors especially neural crest cells.

Furthermore, new susceptibility genes were identified during the past few years. Most of these newly discovered genes were identified using whole exome sequencing, some only in a few family members and some were together with other already known PPGL susceptibility genes. Moreover, using whole exome sequencing, 50% of apparently sporadic PPGLs were identified to have an underlying somatic mutation on the VHL, NF1 and RET genes (41). Identification of an underlying mutation whether germline or somatic, significantly influences current management and follow-up of PPGL. Furthermore, it serves as the platform in the discovery of innovative diagnostic and therapeutic options. Some new mutations associated with PPGL identified were not included in the Cancer Genome Atlas: *H3F3A*, *MDH2*, *PHD1*, *IRP1*, *SLC25A11* and *DLST*. But it should be noted that some of these mutations were found in only a few family members or published as anecdotal case reports (10-13,17-19).

***H3F3A* gene**

Chromatin-remodeling genes mutations indicates the presence of epigenetic modifications in PPGLs (42). *H3F3A* found in chromosome 1 encodes histone H3.3 protein responsible for nucleosome formation. In the study of Toledo *et al.*, a patient carrying a *H3F3A* mutation presented with bilateral pheochromocytoma together with bladder and periaortic paragangliomas (10). This patient also has a history of recurrent tibial giant cell tumor which is similar to another patient carrying the same *H3F3A* mutation with an aggressive retroperitoneal paraganglioma with liver metastases and a history of recurrent and metastatic giant cell tumors.

***MDH2* gene**

A heterozygous variant on exon 4 of *MDH2* was first detected in a patient with multiple metastatic paragangliomas (12). This gene was found to be responsible for encoding malate dehydrogenase enzyme that converts malate to oxaloacetate in the TCA cycle. The study demonstrated a lower *MDH2* activity in mutated tumors but they were unable to document a subsequent accumulation of malate. However, a higher fumarate:succinate ratio was noted indicating fumarate accumulation likely explaining the PPGL development in this patient. Two of the index patient's relatives were found to have the same mutation. Both of them were asymptomatic but one was identified to have the disease due to elevated levels of normetanephrine. *MDH2* germline mutation is present in 0.6% of cases of PPGL with an incomplete penetrance (11).

***PHD1* gene**

PPGLs presenting with polycythemia has been first documented among patients harboring a mutation in *PHD2*, *VHL*, and *HIF2A* genes (38,43,44). Nevertheless, these mutations have been notably absent in a few patients who presented with the similar combination of PPGL and polycythemia, these findings were suggestive of other undiscovered mutations. Later on, in a patient who presented with pheochromocytoma accompanied by polycythemia without an underlying *PHD2*, *VHL*, and *HIF2A* mutations, Yang *et al.* found a new germline mutation in the *PHD1* gene (13). This mutation resulted in the functional loss of prolyl hydroxylase domain protein thereby activating HIF-2 α most likely contributing to pheochromocytoma tumorigenesis. Interestingly, there are some similarities between PPGLs secondary to *HIF2A* and *PHD1* mutations. Both mutations manifest with multiple or recurrent PPGLs predominantly-secreting norepinephrine and presents with polycythemia at an early age. Despite both mutations presenting with polycythemia, erythropoietin is only significantly increased among patients carrying the *HIF2A* mutation in contrast to the mildly elevated level of erythropoietin brought about by the *PHD1* mutation. Moreover, *HIF2A* mutation was found to be associated with duodenal somatostatinoma which has not been documented among patients with *PHD1* mutation.

***ATRX* gene**

ATRX gene located at chromosome X plays a role in the

maintenance of telomeres, chromosome integrity and regulation of transcription (14). In a study by Fishbein *et al.*, it was found that 12.6% of PPGLs carry somatic *ATRX* mutations and most of these patients also possess germline *SDHx* mutations (15). These patients demonstrated clinically aggressive behavior and alternative lengthening of telomeres. In contrast, a more recent study found that only 4% of PPGLs carry *ATRX* mutations (16). But similar to the previous study, half of these patients presented with clinically aggressive PPGLs and most of these patients had a concomitant *SDHx* mutation. This suggests that the identification of an underlying *ATRX* mutation can be used to assess the risk of PPGLs to demonstrate clinically aggressive behavior.

In most studies, *ATRX* mutations occur with another mutation in other known susceptibility genes such as *SDHx* but in a study by Comino-Méndez *et al.*, a patient with an *ATRX* mutation without a coexisting mutation was also presented (45). That patient presented with pheochromocytoma with distant metastases. In the absence of another underlying mutation in the other known PPGL susceptibility genes, the authors concluded that the *ATRX* mutation was most likely the one that caused PPGL occurrence.

IRP1 gene

As described above, despite the identification of multiple genes associated with the syndrome of PPGL presenting with polycythemia, there are still some patients with a similar clinical presentation wherein no genetic mutation was identified. This led to the discovery of IRP1 by Pang *et al.* in a patient demonstrating polycythemia together with pheochromocytoma (17). IRP1 is a cellular iron metabolism regulator. In iron-deficient cells, IRP1 deletion causes a decrease in IRP1 protein levels leading to HIF-2 α stabilization resulting in stimulation of EPO expression (46,47). In this case, Pang *et al.* were able to document a frame shift of exon 3 secondary to a splicing site mutation resulting in a heterozygous IRP1 deletion accounting for the clinical phenotype of polycythemia presenting with PPGL.

SLC25A11 gene

This tumor suppressor gene encodes the mitochondrial 2-oxoglutarate/malate carrier protein participating in the malate-aspartate shuttle. It is mainly involved in the

transport of 2-oxoglutarate from the mitochondrial matrix to the cytoplasm via an electroneutral exchange with malate regenerating the mitochondrial NADH pool to facilitate the function of complex 1 of the electron transport chain (48). *SLC25A11* mutation accounts for approximately 1% of PPGL cases (18). Furthermore, this mutation was associated with a malignant phenotype with 5% of all metastatic patients identified in a cohort of 121 patients have an underlying *SLC25A11* mutation. Five out of seven patients identified to have *SLC25A11* mutation were noted to have a malignant phenotype. It was concluded that this mutation should be considered as a new genetic risk factor which confers a predisposition to metastatic PPGL.

DLST gene

Dihydrolipoamide S-succinyltransferase (*DLST*) is another gene involved in the TCA which is believed to be one of the susceptibility genes for PPGLs. *DLST* is mainly involved in the E2 subunit of mitochondrial α -ketoglutarate dehydrogenase (OGDH) complex which facilitates conversion of α -ketoglutarate to succinyl-CoA and carbon dioxide. Remacha *et al.* detected this mutation in eight unrelated individuals accounting for 6% of patients with apparently sporadic PPGL (19). In terms of PPGL pathogenesis, this mutation is believed to cause the tumorigenesis by interfering with the normal function of the OGDH complex causing an increase in the levels of α -ketoglutarate which subsequently results to a higher α -ketoglutarate to fumarate ratio. It is worth mentioning that the identified individuals with the *DLST* mutation presented with multiple tumors. The identification of this mutation can greatly affect the treatment and monitoring plan for patients harboring *DLST* mutations.

Biochemistry

PPGLs secrete catecholamines which is responsible for the elevated blood pressure and other symptoms or signs such as palpitations, headaches and sweating which better characterize a patient with PPGL. Presenting symptoms of PPGL varies according to the specific catecholamine secreted by a tumor. Norepinephrine can act on both α - and β -adrenoceptors demonstrating a predominant effect on the α -adrenoceptors. α 1-adrenoceptor activation causes vasoconstriction which results to elevation of blood pressure. Sustained hypertension may lead to cerebrovascular accidents, myocardial infarction, renal failure or intestinal

ischemia (49). On the other hand, tumors mainly secreting epinephrine can present with hypotension because of β_2 -adrenoceptor activation causing vasodilation. This vasodilatory effect triggers reflex tachycardia which is typical among patients with an epinephrine-secreting PPGL (50). Dopamine-secreting tumors can also cause hypotension secondary to the vasodilatory effects of dopamine itself but this is very rare and only if plasma dopamine levels are very high. Dopamine-induced stimulation of D1 and D2 receptors may cause diarrhea, nausea and vomiting (51). In line with this, biochemical evaluation is an important step in the diagnosis of every PPGL. It is important to note that not all PPGLs produce and secrete catecholamines; there are tumors which do not produce catecholamines at all and are referred to as biochemically silent. Thus, PPGLs have been divided into four principal biochemical profiles which includes adrenergic, noradrenergic, dopaminergic, and silent (35).

In the past, biochemical evaluation was based on measurement of urine catecholamines and their metabolites such as vanillylmandelic acid and metanephrines (52). At present, it has been well documented from several recent studies that measurement of plasma free metanephrines is more reliable than plasma catecholamines, urinary catecholamines, urinary total metanephrines, urinary vanillylmandelic acid and plasma deconjugated metanephrines (52-55) (Table 2). Particularly, in a recent study by Eisenhofer *et al.* (58), it was found that the measurement of plasma free metanephrines has a higher sensitivity and specificity (96.6% and 94.9%, respectively), compared to measurement of urinary free metanephrines (92.9% and 94.5%, respectively). They also found a significant difference in the sensitivity and specificity of both tests when the patients being tested were classified according to the risk of developing a PPGL. A high risk of developing PPGL was defined by the presence of an adrenal incidentaloma, previous history of PPGL or an underlying mutation in PPGL susceptibility genes. The measurement of plasma free metanephrines was found to be a better screening tool for PPGL than the measurement of urinary fractionated metanephrines among patients with a high risk of having PPGLs with a sensitivity of 96.7% and specificity of 92.8%. As for patients who have a low risk of having PPGL meaning they were tested on the basis of presence of signs and symptoms suggestive of disease without any history of previous disease, adrenal incidentaloma or known susceptibility gene mutation, plasma or urinary fractionated metabolites can be used as a screening tool without

significant difference in their performance. It is important to note that the superiority of plasma free metanephrines will only hold true if an accurate method of measurement, technique and established reference intervals are employed. The US Endocrine Society guidelines recommend the use of liquid-chromatography with electrochemical detection (LC-ECD) or liquid-chromatography with tandem mass spectrometry (LC-MS/MS) in the biochemical evaluation of PPGLs. In addition, it is important to note that measurements of plasma free metanephrines should be facilitated with the patient in a supine position for at least 30 min (59). An upright position may falsely elevate plasma metanephrines level secondary to an increase in norepinephrine/epinephrine release brought about by sympathoadrenal activation. Of note, most laboratory reference values are determined from measurements taken in the upright or seated position. In line with this, laboratories are advised to establish their reference values from data taken in the supine position (60). It is also recognized that age affects the levels of catecholamines and metanephrines. Therefore, it is suggested that age-appropriate levels should be used particularly when the patient belongs in the pediatric age group (61).

The process by which we measure levels of catecholamine and its metabolites has also changed throughout the years. Traditional bioassays, colorimetric, fluorometric techniques and radioenzymatic assays were used in the past for the biochemical diagnosis of PPGL but our technology have progressed to the use of LC-ECD or LC-MS/MS. With the availability of these more advanced techniques, measurement of fractionated metanephrines was made possible which is an important improvement over measurements of total metanephrines using the colorimetric or fluorometric technique (61). The level of plasma metanephrines using enzyme immunoassay was found to be suboptimal compared to levels measured by LC-MS/MS (56,62,63). A higher sensitivity and specificity for the diagnosis of PPGLs were also noted using LC-MS/MS making it the current method of choice for plasma free metanephrines measurement (56,63). In addition, the use of LC-ECD and LC-MS/MS enabled the measurement of methoxytyramine levels which is a dopamine metabolite (61). Measurement of methoxytyramine in addition to the standard panel of plasma free metanephrines has been shown to increase the detection of PPGLs modestly because it allows the identification of tumors that exclusively produce dopamine. In addition to metanephrine and normetanephrine, methoxytyramine measurement significantly increased the

diagnostic sensitivity for head and neck paragangliomas (HNPGs) from 22.1% to 50% (57). This signifies the importance of determining methoxytyramine levels among patients suspected or with known HNPG. One of the difficulties encountered in the biochemical diagnosis of PPGLs is the interpretation of results under the influence of medications which can interfere with analysis (60,64). A number of patients who would require biochemical testing are usually on antihypertensive medications which can also affect analysis. The use of LC-ECD was found to be susceptible to analytical interference with the use of several medications including antihypertensive drugs such as phenoxybenzamine and α -adrenoceptor blockers (65-67). One of the advantages of using LC-MS/MS is its lack of interference from antihypertensive drugs such as α -adrenoceptor blockers, diuretics and ACE inhibitors (68). Using this technique in the measurement of plasma metanephrines enables the patients to continue their current antihypertensive regimen decreasing the risk of sudden elevations of blood pressure. In addition, the use of mass spectrometry also provides easier workflows, short chromatography run-times and higher throughput (69).

As mentioned earlier, the excess levels of catecholamines among patients with PPGLs may cause hypertensive crisis and in severe cases may also cause end organ damage in the form of myocardial infarction, cerebrovascular accident or multiple organ failure especially during stressful conditions particularly while ongoing a surgical procedure. PPGLs possess an excessive amount of catecholamines and its release may be elicited by induction of general anesthesia, abdominal pressure fluctuation as well as direct tumor handling during the procedure itself. With the increased risk for adverse cardiovascular events, preoperative blockade using α - and β -adrenoceptor blockers and calcium channel blockers is very highly recommended (60,70,71). Preoperative adrenoceptor blockade has been shown to prevent complications such as major adverse cardiovascular events pre- as well as peri-operatively (72). Although current recommendations by the US Endocrine Society guidelines strongly advocate the use of preoperative blockade (60), there are other groups that question the use of pre-operative adrenoceptor blockade because they were unable to document a significant difference in the incidence of hypertensive episodes and major complications between patients who underwent preoperative blockade and those who did not (73). A recent review has also been released which states that there is a low risk of decompensation prior to surgery for patients not on pre-

operative blockade without documented bad outcomes despite intermittent blood pressure elevations. They further stated that given this data, surgical treatment may be facilitated without delay which is sometimes secondary to the need to initiate preoperative adrenoceptor blockade (74). This approach is considered as erroneous by our group and at least in US could lead to very complicated situations like when a patient deteriorates shortly before an operation (e.g., sudden hypertensive crisis or tachyarrhythmia resulting in stroke or other complications) or when a physician could be investigated for inappropriate clinical care based on current US guidelines and recommendations (60,70,71,74). This scenario is well summarized by Wolf *et al.* (75) who pointed out that unexpected situations do occur not only during surgery but before as well, which may significantly increase the probability of an adverse event, hence, there is a need for physicians to be always aware of the risks involved and safeguard patients from these catastrophic events. In addition, our group recognizes well the risk of potential unforeseen adverse events among patients with PPGL and if this adverse outcome indeed happens without the use of preoperative adrenoceptor blockade, physicians may be at risk for ethical issues and may even be accused of medical malpractice or negligence.

Immunohistochemistry

Despite the availability of genetic testing to identify an underlying driver mutation for individuals with PPGL, it is laborious and expensive, especially in some countries. To resolve this issue, immunohistochemistry can be used as a guide to limit time and expenses required to identify the underlying mutation (76). In addition, immunohistochemistry can also be used to assess the pathogenicity of variants of uncertain significance identified using genetic testing (77) (Table 3).

Immunohistochemistry identifies *SDHx* mutations by loss of SDHB or SDHA protein expression. Among individuals with *SDHB*, *SDHC*, and *SDHD* mutations, their tumors are negative for SDHB immunostaining and positive for SDHA immunostaining in contrast to individuals with *SDHA* mutation only who are negative for both SDHB and SDHA immunostaining (77,79,82) (Table 3). SDHB immunohistochemistry have a strong correlation with an underlying *SDHx* subunit gene mutation with a sensitivity and specificity of 95.0% and 81.8%, respectively (79). Interobserver variation analysis using SDHB/SDHA immunohistochemistry was assessed and it was noted

that sensitivity for this approach ranged from 83.58% to 98.57% with a mean of 94.23%; specificity ranged from 74.03% to 96.11% with a mean of 84.35% (77). Although SDHB/SDHA immunohistochemistry is a useful tool in the identification of PPGLs associated with a *SDHx* mutation, there are instances when there is disagreement in the interpretation of a weakly diffuse SDHB immunostaining. This weak diffuse signal is usually observed among patients with *SDHD* mutations (83). Tumors with an underlying *SDHD* mutation present with a diffuse cytoplasmic blush which could be mistakenly read as a granular cytoplasmic staining characteristic of a non-*SDHx* related tumor (84). When a weak diffuse SDHB immunostaining is erroneously read as positive, we miss identifying an individual with a *SDHx* mutation compromising patient care. To resolve this issue, SDHD immunostaining was proposed as a complementary tool. Interestingly, patients with *SDHx* related PPGLs are positive on SDHD immunostaining as compared to non-*SDHx* related tumors that are negative on SDHD immunostaining (78). The SDH complex has a catalytic and anchoring component. SDHA and SDHB forms the catalytic component while SDHC and SDHD functions as an anchor which connects the complex to the inner mitochondrial membrane (84). It is hypothesized that because SDHB and SDHA protein levels are also low in non-*SDHx*-related PPGLs, SDHC and SDHD protein levels are low too causing the SDHD protein to be masked, thereby, demonstrating a negative SDHD immunostaining. On the other hand, among *SDHx*-related tumors, there is disruption of the active complex that uncovers SDHD protein resulting in a positive SDHD immunostaining. This finding is particularly useful in verifying possible false positives in tumors presenting with a weak diffuse SDHB immunostaining. A negative SDHD immunostaining together with a weak diffuse SDHB immunostaining indicates a non-*SDHx* related tumor while a positive SDHD immunostaining in the presence of a weak diffuse SDHB immunostaining is indicative of a *SDHx*-related tumor (78).

As mentioned, *FH* mutation leads to a syndrome of hereditary leiomyomatosis and renal cell carcinoma together with pheochromocytoma or paraganglioma. It has been shown that leiomyomas and renal cell cancer with *FH* mutation leads to a loss of *FH* protein expression (34). Given that there is only a small subset of patients with PPGL with an underlying *FH* mutation, studies on immunohistochemistry of these tumors are limited. In a study by Udager *et al.*, a patient with retroperitoneal mass with an underlying *FH* mutation revealed a loss of

cytoplasmic *FH* immunohistochemistry staining (79).

Carbonic anhydrase 9 (CA9) preserves normal intracellular and extracellular pH by stimulating carbon dioxide hydration. It is recognized as a hypoxia-induced gene and was found to be increased in renal clear cell carcinoma which is associated with a malfunctioning VHL-hypoxia inducible factor pathway (85). In line with this, CA9 is strongly expressed in pheochromocytomas with an underlying *VHL* mutation (86). Favier *et al.* were able to demonstrate a positive CA9 immunostaining for 42 out of 48 (88%) patients with *VHL* mutation (81). Immunostaining for CA9 was also found to be negative for 144 out of 159 (91%) patients without a *VHL* mutation. They proposed that CA9 immunohistochemistry could be tested in all PPGLs to identify underlying germline or possibly somatic *VHL* mutation. This technique can also be used to assess the pathogenicity of *VHL* variants of uncertain significance identified using genetic testing.

It is known that PPGLs are made of chromaffin cells. Sustentacular cells which are stained using S100 protein are almost always present among PPGLs. In a more recent study, it has been found that in addition to sustentacular cells, CD163 and CD68 positive cells were identified indicating the presence of cells with monocyte-macrophage lineage but they were unable to find a significant association of monocyte to sustentacular cell ratio and genotype or location of tumor. Despite this lack of association, identification of these monocytes within PPGLs can serve as a catalysis for the development of new treatment modalities (80).

Metabolomics

According to the Warburg hypothesis, cancer is caused by an impairment in the mitochondrial respiratory function causing a shift from oxidative phosphorylation to glycolysis. A number of signaling and metabolic pathways are activated secondary to this cellular respiration shift which promotes proliferation and prolongs survival of cells (87). As described above, some cases of PPGLs are known to be caused by mutations in genes encoding proteins involved in the Krebs cycle. Mutation in the genes encoding *SDHx*, *FH*, *MDH2*, and *IDH* predispose an individual to develop hereditary PPGLs. A pathogenic mutation in one of these gene leads to accumulation of succinate, fumarate, or 2-hydroxyglutarate which causes α -ketoglutarate dependent enzyme inhibition resulting to pseudohypoxia and hypermethylation which is responsible for tumorigenesis (88). Using new technologies, nuclear magnetic resonance spectroscopy and gas chromatography-

Table 1 Newly discovered genes in the pathogenesis of PPGL

Gene	Date of discovery	Gene role	Clinical presentation	Gene type	References
<i>H3F3A</i>	2016	Encodes histone H3.3 protein responsible for nucleosome formation	Bilateral pheochromocytoma, bladder and periaortic paraganglioma, giant cell tumor of bones	Somatic	(10)
<i>MDH2</i>	2015	Encodes mitochondrial malate dehydrogenase which converts malate to oxaloacetate	Multiple, noradrenergic, metastatic	Germline	(11,12)
<i>PHD1</i>	2015	Functional loss prolyl hydroxylase domain protein causing activation of HIF-1 α and HIF-2 α	Multiple, recurrent, noradrenergic, early onset of polycythemia	Germline	(13)
<i>ATRX</i>	2015	Encodes transcriptional regulator ATRX which plays a role in the maintenance of telomere, chromosome integrity and transcription regulation	Clinically more aggressive, metastatic	Somatic	(14-16)
<i>IRP1</i>	2018	Encodes IRP1 involved in cellular iron metabolism and negative regulation of HIF-2 α protein translation	Pheochromocytoma, noradrenergic, polycythemia	Somatic	(17)
<i>SLC25A11</i>	2018	Encodes mitochondrial 2-oxoglutarate/malate carrier involved in the malate-aspartate shuttle	Metastatic	Germline	(18)
<i>DLST</i>	2019	Encodes E2 subunit of mitochondrial α -ketoglutarate dehydrogenase (OGDH) complex	Multiple, noradrenergic	Germline	(19)

PPGL, pheochromocytomas and paragangliomas.

Table 2 Summary of studies on the recent advances in the biochemistry of PPGLs

Authors	Journal	Year	Findings
Weismann <i>et al.</i> (56)	<i>European Journal of Endocrinology</i>	2015	Higher sensitivity and specificity of plasma metanephrines measurement using LC-MS/MS
Rao <i>et al.</i> (57)	<i>European Journal of Endocrinology</i>	2017	Measurement of plasma methoxytyramine enables the detection of PPGLs which exclusively secrete dopamine Inclusion of methoxytyramine to the standard biochemical test panel yields better diagnostic utility
Eisenhofer <i>et al.</i> (58)	<i>Clinical Chemistry</i>	2018	Plasma free metabolite is a better diagnostic test for PPGLs than urinary fractionated metabolites with a higher sensitivity and specificity

PPGL, pheochromocytomas and paragangliomas.

mass spectrometry or liquid chromatography-mass spectrometry can now be used to profile and quantify the resulting metabolite accumulation brought by these pathogenic mutations (89). This is especially important in any situation when genetic testing yields unresolved results wherein in a variant of uncertain significance may

be identified. In these instances, metabolomic data of tumor tissue can help verify functionality of the underlying germline or somatic variants. Thus, identification and quantification of metabolites accumulated in a tumor can be used to uncover new PPGL susceptibility genes which in turn could serve as a new platform for novel diagnostic and

Table 3 Summary of studies on the recent advances in the immunohistochemistry of PPGLs

Authors	Journal	Year	Findings
Papathomas <i>et al.</i> (77)	<i>Modern Pathology</i>	2015	SDHB/SDHA immunohistochemistry reliably identifies patients with <i>SDHx</i> mutations and can be used to evaluate variants of unknown significance for pathogenicity
Menara <i>et al.</i> (78)	<i>Journal of Clinical Endocrinology and Metabolism</i>	2015	SDHD immunostaining can be used to predict or validate <i>SDHx</i> gene variants particularly if there is a weak diffuse SDHB immunostaining
Udager <i>et al.</i> (79)	<i>Human Pathology</i>	2018	Confirmation of the utility of SDHB immunostaining in the identification of <i>SDHx</i> mutations First report of FH-deficient immunostaining in a patient with known PGL and <i>FH</i> germline mutation
Farhat <i>et al.</i> (80)	<i>Endocrine Pathology</i>	2019	Identification of CD163 and CD68 positive cells indicative of a monocyte-macrophage lineage in PPGLs
Favier <i>et al.</i> (81)	<i>Modern Pathology</i>	2019	Use of CA9 immunostaining in the identification of <i>VHL</i> mutation

PPGL, pheochromocytomas and paragangliomas.

Table 4 Summary of studies on the recent advances in the metabolomics of PPGLs

Authors	Journal	Year	Findings
Richter <i>et al.</i> (92)	<i>Journal of Clinical Endocrinology and Metabolism</i>	2014	Succinate:fumarate ratio and other metabolites measured using mass spectrometry can be used as a tool to identify underlying <i>SDHx</i> mutations
Imperiale <i>et al.</i> (93)	<i>Neoplasia</i>	2015	HRMAS NMR spectroscopy can be used for metabolome profiling of PPGLs
Kim <i>et al.</i> (94)	<i>Molecular Genetics and Metabolism Reports</i>	2016	Higher succinate:fumarate ratio in PPGLs, gastrointestinal tumors and renal cell carcinoma of SDH-deficient individuals compared to their SDH-sufficient counterparts
Richter <i>et al.</i> (91)	<i>Genetics in Medicine</i>	2019	Use of succinate:fumarate ratio, fumarate:malate ratio and level of 2-hydroxyglutarate to identify pathogenic variants of <i>SDHx</i> , <i>FH</i> and <i>IDH</i> mutations, respectively

PPGL, pheochromocytomas and paragangliomas.

therapeutic options. Accumulation of certain metabolites can also be used as a marker for metastases and monitoring of therapeutic response (90-92) (Table 4).

SDHx mutations result in succinate accumulation. The resulting higher succinate:fumarate ratio enables differentiation from non-*SDHx* PGLs making it a very useful metabolic marker (90,94,95). Tumor tissue succinate:fumarate ratios can recognize *SDHx*-related PPGLs with a sensitivity and specificity of 93% and 97%, respectively which is similar to the performance of other available diagnostic tests (92). Interestingly, quantification of succinate as a screening test for *SDHx*-related PPGLs resulted in 100% sensitivity and specificity using a threshold of 0.253 nmol/mg to distinguish them from non-*SDHx* PPGLs and 0.096 nmol/mg to differentiate them from sporadic PPGLs (93).

Succinate:fumarate ratio was also found to be higher in metastatic disease as compared to non-metastatic disease (92). This highlights the potential of succinate:fumarate ratio as a marker for metastatic disease. Furthermore, the measurement of succinate:fumarate ratio, especially in plasma, could also be used to monitor PPGL patients and their therapeutic response including any recurrence or new metastasis (90).

Imaging

The accessibility of new functional imaging modalities have added value in accurate diagnosis of PPGLs (96). As recommended by the US Endocrine Society Guidelines, a confirmatory biochemical evidence of disease is required before contemplating on any imaging tool in PPGLs (60).

Table 5 Summary of radiopharmaceuticals and proposed recent algorithm for nuclear imaging of PPGLs (101)

Tracer	Target receptor	First choice	Second choice	Third choice (if [¹⁸ F]FDOPA or [⁶⁸ Ga]Ga-SSA is not available)
[¹²³ I]MIBG	Norepinephrine transporter	PHEO (sporadic)	Inherited PHEO (except <i>SDHx</i>): <i>NF1/RET/VHL/MAX</i>	Extra-adrenal sympathetic and/or multifocal and/or metastatic and/or <i>SDHx</i> mutation
[¹⁸ F]FDOPA	Large, neutral amino acid transporter system L	PHEO (sporadic) Inherited PHEO (except <i>SDHx</i>): <i>NF1/RET/VHL/MAX</i>	HNPGL (sporadic) Extra-adrenal sympathetic and/or multifocal and/or metastatic and/or Halics mutation	
[⁶⁸ Ga]Ga-DOTATOC /DOTATATE/ DOTANOC	Somatostatin receptors	HNPGL (sporadic) Extra-adrenal sympathetic and/or multifocal and/or metastatic and/or <i>SDHx</i> mutation	PHEO (sporadic) Inherited PHEO (except Halics): Halrs	
[¹⁸ F]FDG	GLUTs		Extra-adrenal sympathetic and/or multifocal and/or metastatic and/or <i>SDHx</i> mutation	PHEO (sporadic) Inherited PHEO (except <i>SDHx</i>): <i>NF1/RET/VHL/MAX</i> Extra-adrenal sympathetic and/or multifocal and/or metastatic and/or <i>SDHx</i> mutation

PPGL, pheochromocytomas and paragangliomas; PHEO, pheochromocytoma; GLUTs, glucose transporters.

Computed tomography (CT) and magnetic resonance imaging (MRI) are the recommended initial anatomic imaging modalities after biochemical confirmation (96,97). After lesion determination using CT or MRI, the decision whether additional whole-body anatomic and functional studies are necessary depends on the biochemical profile, tumor size, as well as likelihood for metastasis. Patients who present with extra-adrenally located lesions greater than 5 cm, secreting norepinephrine/normetanephrine or dopamine/methoxytyramine, will more likely benefit from such imaging to further define extent of disease due to a higher metastatic potential (97). All patients with *SDHB*-related PPGL and those in whom surgical specimen of tumor tissue detected *ATRX* and *HIF2A* mutations and primary multiple PPGLs, will also benefit from those studies (39,98).

***Iodine-123-metaiodobenzylguanidine* (¹²³I-MIBG) scintigraphy**

With regards to the functional imaging approaches, ¹²³I-metaiodobenzylguanidine (¹²³I-MIBG) scintigraphy is

currently being utilized for imaging of pheochromocytomas believed to be sporadic (99). Aside from being extensively available and relatively inexpensive, high-quality images and relatively low radiation exposure are some of its advantages (100). Abnormal findings on ¹²³I-MIBG scintigraphy include a noticeable increased adrenal uptake with concomitant gland enlargement as well as focal uptakes outside the adrenal tissue without normal physiologic distribution (101) (*Table 5*). A number of studies on pheochromocytoma patients have documented sensitivity and specificity of ¹²³I-MIBG scintigraphy at 83–100% and 95–100%, respectively. However, lower sensitivity of 52–75% was found in patients having extra-adrenal, multiple, recurrent, and hereditary PPGLs. Nonetheless, it is of great use in metastatic disease when radiotherapy using ¹³¹I-MIBG is planned or when there is an increased risk of metastasis and recurrence due to a large size of primary or extra-adrenal tumor (96,99).

Expression of several transporters and receptors by PPGLs such as norepinephrine transporter, glucose transporter (GLUT), amino acid transporter, and somatostatin receptor (SSTR) has given much attention to

novel nuclear imaging modalities targeting such receptors (Table 5). The following positron emission tomography hybridized with CT (PET/CT) radiopharmaceuticals are presently being employed in disease evaluation, treatment plan, and assessment of tumor response: ^{18}F -fluorodeoxyglucose (^{18}F -FDG), ^{18}F -fluorodopa (^{18}F -FDOPA), and ^{68}Ga -tetraazacyclododecanetetraacetic acid (DOTA) analogs (96,101).

^{68}Ga PET/CT SSTR analogues

PET/CT imaging with ^{68}Ga -labeled DOTA peptides such as DOTA(0)-Tyr(3)-octreotate (DOTATATE), DOTA(0)-Phe(1)-Tyr(3)-octreotide (DOTATOC), and DOTA(1)-Nal(3)-octreotide (DOTANOC) bind to SSTR-expressing tumors more efficiently and has quickly evolved as the favored functional imaging modality for PPGLs in general (97,101). More specifically, DOTATATE has greater affinity for SSTR2 receptors. In a recent study by Archier *et al.*, a higher sensitivity of 93% for ^{68}Ga -DOTATATE PET/CT compared to ^{18}F -FDOPA PET/CT (89%), and conventional imaging (76%) was observed in terms of lesion detection and localization. This has been mainly credited to its high tumor-to-background uptake ratio (102). Moreover, ^{68}Ga -DOTATATE PET/CT have shown superiority over other functional imaging scans in improved detection of sporadic, *SDHB*-metastatic disease, as well as primary and *SDHD*-related HNPGLs (97,102-104). Aside from its significant role in primary tumor detection, it can further identify patients eligible for peptide receptor radionuclide therapy (PRRT) with radiolabeled SSTR agonists (105).

SSTR antagonists

One of the latest developments concerning SSTR targeting is the introduction of SSTR antagonists (^{111}In -DOTA-BASS, ^{111}In -DOTA-JR11, Ga-DOTA-JR11, Ga-NODAGA-JR11). Preclinical and clinical studies at present have shown evidence of greater tumor uptake, despite lack of internalization, which may improve image quality and increase lesion detection by SSTR PET/CT (101,105). When compared to an SSTR agonist in the use of human specimens, ^{125}I -JR11 (an SSTR antagonist) have shown increased avidity to SSTR2 sites resulting to better tumor localization and value of treatment approach using radiolabeled SSTR antagonists. Aside from pheochromocytoma, other tumors that can be explored by imaging using SSTR2 antagonists are breast cancer,

small cell lung cancer, renal cell cancer, non-Hodgkin's lymphomas, and medullary thyroid cancer (105).

^{18}F -fluorodopa PET/CT

The application of ^{18}F -FDOPA PET/CT in the imaging of L-type amino acid transporters (mainly LAT1 and LAT2) it a well-accepted imaging tool to spot pheochromocytomas (101). It has a sensitivity of up to 100% for primary pheochromocytomas equivocal on ^{123}I -MIBG scintigraphy (106). Also, it is an excellent diagnostic tool for HNPGLs, but with lower sensitivity for retroperitoneal paragangliomas related to *SDHx* mutations. In terms of metastatic disease, it offers higher lesion detection rate in *SDHB*-negative patients than in *SDHB*-positive ones (100).

Whereas ^{68}Ga -DOTATATE PET/CT do better than other imaging techniques in the evaluation of PPGLs, its high uptake by the healthy adrenal glands poses a problem in the discovery of small pheochromocytomas associated. On the other hand, ^{18}F -FDOPA has the advantage of detecting these small lesions related to gene mutations that result in multiple endocrine neoplasia type 2 (MEN2), neurofibromatosis type 1 (NF1), von Hippel-Lindau (VHL) and other paraganglioma (PGL) syndromes due to its very low physiologic uptake in the normal adrenal medulla (101,102). This imaging modality also helps in the assessment of patients with *MAX* mutations developing bilateral/multiple tumors in the same gland (36). Moreover, it was found to surpass ^{68}Ga -DOTATATE in identifying other primary tumors and/or metastases in somatic *HIF2A* mutation patients. Furthermore, ^{18}F -FDOPA can be utilized for HNPGL detection in the absence of ^{68}Ga -DOTATATE with a sensitivity and specificity of >90% and 95–100% respectively (36).

^{18}F -Fluorodeoxyglucose PET/CT

^{18}F -FDG PET/CT is a widely accepted imaging tool in oncology from disease diagnosis to monitoring of treatment response. It has an estimated overall average sensitivity and specificity of 84% and 88% respectively, in cancer (107). Aside from it being generally available, nuclear imaging with ^{18}F -FDG PET/CT is preferred in an established metastatic disease. It has also been suggested for the localization and prognosis of highly aggressive NETs and non-NETs (106). In particular, its acceptable sensitivity in metastatic PGLs mainly related to *SDHx* mutation has been well recognized which is in contrary to ^{18}F -FDOPA

PET/CT (96,100,101,103,106). Though ^{123}I -MIBG scintigraphy is a radiotherapeutic option for metastatic PPGLs, unimpressive detection rates when it comes to tumors related to *SDHB* has led to an enhanced application of ^{18}F -FDG PET/CT and other newly developed functional imaging in the evaluation of biochemically proven hereditary PPGLs (108,109). Currently, ^{18}F -FDG PET/CT present a reasonable and readily accessible imaging option for patients with *SDHx*-related tumors, extra-adrenal PPGLs, with multifocality/metastases (101,109).

Treatment

There has been no standardized protocol in the treatment of metastatic PPGL and that prospective studies are lacking due to its rarity. Despite ongoing developments in treatment, factors determining prognosis and survival are yet to be defined (98). Genetic predilection, tumor load, biochemical phenotype, disease progression, and presence of metastases primarily determine disease prognosis (110). More specifically, better prognosis was observed in the following variables: young age, female gender, aortic/carotid body tumors, complete surgical resection, local disease, and presence of second primary malignancy (98). Moreover, better survival was associated with HNPGLs, metanephrines less than five-fold the upper limits of normal range, and low proliferative index (5). On the other hand, reduced survival rate and poor prognosis were more correlated to male sex, older age at time of diagnosis, larger primary tumor size, high dopamine levels, incomplete resection, and presence of distant metastasis (6,111). Both localized (radiotherapy, radiofrequency, or cryoablation) and systemic therapies (chemotherapy or molecular targeted therapies) are available therapeutic options when surgery is not generally feasible (110). Specifically, in terms of localized treatment approach, ablative therapy in metastatic PPGL can successfully result in palliation of symptoms and decrease in tumor burden (112). Currently, there has been an increasing interest on the value of radionuclide therapy for NETs - ^{131}I -MIBG and the very recent PRRT (110).

Radionuclide therapy implicates the use of radiolabeled peptides with high affinity to the involved receptor. In addition to surgery and chemotherapy, ^{131}I -MIBG treatment has been effectively used in the management of malignant and metastatic PPGLs. ^{177}Lu -DOTATATE (Lutathera[®]) therapy has been recently used in metastatic PPGLs (113,114).

^{131}I -MIBG therapy

Treatment with high-specific activity ^{131}I -MIBG (Azedra[®]) has been very recently being employed to treat patients with metastatic PPGLs including those with unsatisfactory outcomes even after conventional therapies (115,116). The beta particles from this radionuclide are responsible for damaging the cells while the gamma ray permits dosimetry calculations. Using the high specific activity formulation (2,500 mCi/mg) allows better targeting and higher tumor concentration (117). It has become the first systemic radiotherapy for metastatic PPGLs approved by United States Food and Drug Administration (FDA) in July 2018 (115). In a study by Gonias *et al.*, overall response and survival rates of 57% and 64% respectively. Furthermore, *SDHB* patients were observed to achieve complete/partial response more likely and that prior chemotherapy or radiation were poor predictors of survival (118). Data from a pivotal phase II open-label, multicenter trial involving patients with MIBG-positive metastatic PPGLs showed that within 12 months after treatment with Azedra, prolonged blood pressure control with partial response and stable disease in 92% of patients were significantly observed (116). The most common observed adverse effects were myelosuppression, nausea, vomiting, fatigue and dizziness (106,115).

Peptide receptor radionuclide therapy (PRRT)

^{177}Lu -DOTATATE (Lutathera[®]) has been recently approved in the United States for treatment of NETs (106). A recent retrospective study by Vyakaranam *et al.* presented positive outcomes of ^{177}Lu -DOTATATE as first-line therapy or for progressive PPGL, after showing evidence of increased overall survival with better scintigraphic (>50%) and biochemical responses (>50% decrease) (119). Moreover, in a study of 20 PPGL patients by Kong *et al.*, partial and stable disease was noted in 29% and 62% of patients respectively, 3 months after ^{177}Lu -DOTATATE treatment (113). In a study by Yadav *et al.*, capecitabine with concomitant ^{177}Lu -DOTATATE treatment in malignant PGL achieved partial tumor response and stable disease in 28% and 56% of patients respectively. A decrease in chromogranin A levels was also observed in 28% of patients (114). Despite evidence of treatment response, modified protocols using longer radioisotope infusion time and lesser administered dose are being utilized to prevent severe adverse reactions such as catecholamine crisis and tumor lysis syndrome (120).

Chemotherapy

An existing management for advanced PPGLs includes chemotherapy with combination regimen of cyclophosphamide/vincristine/dacarbazine (CVD). In 50% of cases, CVD therapy causes relief of symptoms and tumor regression but only temporary (121). Temozolomide (TMZ), a novel alkylating agent, has been utilized as an alternative chemotherapeutic agent to dacarbazine in various types of tumor (121). Moreover, it has been proposed to be more efficient in *SDHB*-related metastatic PPGLs, inferring that PPGLs are effectively targeted depending on genetic background (122). Poly (ADP-ribose) polymerase (PARP) is an enzyme which prevents DNA breakage and instability by producing ADP-ribose-conjugated PARP (PADPR). Since most chemotherapeutic agents including TMZ initiate DNA-damaging effects, combination with PARP inhibitors can be an effective treatment strategy for metastatic tumors (121). Findings in a recent experimental study by Pang *et al.* have shown improved therapeutic effects of genotoxic agents, lesion reduction, and prolonged overall survival by combining PARP inhibitor olaparib with TMZ targeting the nicotinamide adenine dinucleotide (NAD⁺)/PARP DNA repair pathway in *SDHB*-mutated experimental pheochromocytoma cells (121).

BEZ235 is another chemotherapeutic agent recently being studied for treatment of malignant pheochromocytoma. It generates anti-tumoral effects by inhibiting both phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin complex 1 or 2 (mTORC1/2) kinase activity through attachment to the ATP-binding part of these enzymes (123,124). In vivo study on rat pheochromocytomas by Lee *et al.* have shown that treatment with BEZ235 resulted to a decline in norepinephrine transporter expression by inducing cytotoxic and anti-proliferative effects (125). Altogether, PI3K/mTOR inhibition has demonstrated induction of cell apoptosis with significant reduction in cell proliferation and angiogenesis contributing to the response of experimental pheochromocytoma cells (125).

²²³Radium dichloride (Xofigo)

Radium chloride (Ra) for treatment of patients with castration resistant metastatic prostate cancer became available in 2013 (126). Patients who may benefit from ²²³Ra-chloride are selected through bone scintigraphy and the activity administered is calculated based on the patient's

weight and not the degree of accumulation of activity on the diagnostic scan (126). In a case report by Makis *et al.* in 2016, ²²³Ra treatment in a patient with *SDHB* positive malignant hereditary PPGL syndrome improved pain control and ambulation (127). The primary treatment-related adverse event is myelosuppression including anemia, thrombocytopenia, and neutropenia (128). However, future prospective studies are yet to be needed to establish its efficacy and therapeutic benefits in metastatic PPGL bone metastases.

Other therapies including immunotherapy

In cases of malignant pheochromocytomas refractory to both radiation therapy and chemotherapy, tyrosine kinase inhibitors such as Sunitinib, appear to be beneficial. Sunitinib, FDA-approved for the treatment of pancreatic NETs, displays both antiproliferative and antitumor effects through suppression of tumor angiogenesis by inhibiting vascular endothelial growth factor (VEGF) as well as direct inhibition of catecholamine synthesis and secretion (129). In a retrospective study involving metastatic PPGL patients, 47% demonstrated partial response, stable disease, with improvement in blood pressure and performance status after Sunitinib treatment (130). Furthermore, in a recent study using immunotherapy in a pheochromocytoma mouse model, intratumoral injections of mannan-BAM, toll-like receptor ligands, and anti-CD40 led to tumor volume stabilization, reduction in liver metastatic lesions, and longer median survival (131).

Until now, inadequate treatment options are available for metastatic PPGLs. Chemotherapy and radionuclide therapy as conventional therapeutic approach offer symptom relief and biochemical control however achieve less in terms of increasing survival. In the future, novel treatment strategies might be more effective recognizing the value of specific signaling pathways and molecular targets responsible for the development of malignant PPGL.

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Footnote

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