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Pheochromocytoma: A genetic and diagnostic update

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Abstract

Objective: Pheochromocytomas and paragangliomas (PPGLs) are neuroendocrine tumors derived from adrenal or extra-adrenal locations, respectively. Most PPGLs synthesize and secrete catecholamines and their metabolites. A majority of PPGLs are hereditary, and cause unspecific symptoms related to catecholamine excess such as palpitations, uncontrolled hypertension, diaphoresis, headaches, shortness of breath, and anxiety. Upon suspicion of PPGL, specific metabolomic, molecular, biochemical, imaging, and histopathological studies are performed to prove, localize, treat, and monitor disease progression. Recently, improved diagnostic tools allow physicians to accurately diagnose PPGL, even in patients presenting with small (less than 1 cm) or biochemically silent tumors, which previously delayed proper detection and treatment.

Methods: This review outlines the most updated approach to PPGL patients, and presents a new diagnostic approach for physicians in order to increase earlier tumor identification and accurately assess metastatic behavior.

Conclusion: We presented the most recent advances in genetics, epigenetics, metabolomics, biochemical, and imaging diagnoses of this rare tumor in order to properly assess disease, decide treatment options, and manage follow-up. We also elaborated new therapeutic perspectives in these very rare neoplastic entities.

Introduction

Pheochromocytomas and paragangliomas (PPGLs) are chromaffin cell neuroendocrine tumors characterized by catecholamine synthesis, release, and metabolism in the adrenal medulla or extra-adrenal sympathetic or parasympathetic paraganglia, respectively (1). With a prevalence of 0.1 – 0.6% in hypertensive adults and an annual general population incidence of 1 per 100,000, clinical suspicion remains the primary indicator for initiating a biochemical workup to confirm a diagnosis of PPGL (2).

Patients most commonly present with recurring episodes of hypertension, palpitations, profuse diaphoresis, and pallor (3, 4). Less frequently, PPGL may manifest as nausea, vomiting, flushing, and weight loss. Recently, Pourian et al. established a clinical symptomatology likelihood ratio, which found that only palpitations, headache, and diaphoresis are significant when considering a possible PPGL diagnosis (5). Although the general symptoms remain non-specific, catecholamine-induced organ damage, particularly to the endocrine, metabolic, and cardiovascular systems should indicate a full diagnostic workup for PPGL.

While improper or delayed PPGL diagnosis has long been associated with the destructive results of a sudden release in catecholamines resulting in adverse cardiovascular events (6, 7), Ferreira et al. performed the first prospective study on the extent of cardiac involvement in PPGL

patients (8). Unlike previous reports detailing the reversal of catecholamine-induced myocarditis following removal of the unsuspected tumor (9), Ferreira discovered persistence of subclinical systolic and diastolic dysfunction in addition to markers for focal and diffuse fibrosis, as well as cardiac abnormalities distinct from the hypertensive control subjects used throughout the study {Ferreira, 2016 #11021}. In the future, early identification of cardiac catecholamine toxicity could be used to predict potentially disastrous cardiovascular events and prevent long-term myocardial alterations (8).

The present review highlights the latest genetic, metabolomic, biochemical, and functional imaging approaches to update the diagnostic algorithm of hereditary and sporadic PPGLs.

Genes involved in PPGL tumorigenesis

A majority of PPGLs can be ascribed to either germline (~40%), or somatic mutations (~30%) (10, 11, 12). Mutations in more than 19 genes involved in tumorigenesis were discovered in PPGLs, of which at least 12 are associated with a syndromic PPGL presentation (**Table 1**). PPGL susceptibility genes are divided into two clusters, Cluster 1, pseudohypoxic, and Cluster 2 rich in kinase signaling.

Cluster 1 breaks into Cluster 1a, which contains genes encoding Krebs cycle enzymes – mitochondrial succinate dehydrogenase complex (*SDHx*: *SDHA*, *SDHB*, *SDHC*, *SDHD*), the SDH complex assembly factor 2 (*SDHAF2*), fumarate hydratase (*FH*), malate dehydrogenase 2 (*MDH2*), and isocitrate dehydrogenase (*IDH*), and Cluster 1b consisted of the von Hippel-Lindau (*VHL*) tumor suppressor gene, hypoxia-inducible factor 2 α (*EPAS1/HIF2A*), egl-9 family hypoxia inducible factor 2 (*EGLN2/PHD1*) and factor 1 (*EGLN1/PHD2*); reviewed in (12).

Tumors harboring mutations in genes from Cluster 1 are highly vascularized and overexpress vascular endothelial growth factor (VEGF) and VEGF receptors (13), which is a part of the pseudohypoxic signature.

Patients with somatic mutations in *EPAS1/HIF2A* present with a new syndrome of PPGLs with or without erythrocytosis and/or somatostatinomas (14, 15). Novel *EGLN1* and *EGLN2* mutations were also identified in patients presenting with PPGLs and erythrocytosis without *EPAS1/HIF2A* mutations (16).

PPGLs associated with the second cluster, are comprised of genes involved in kinase receptor signaling: *RET* protooncogene, neurofibromin 1 (*NF1*), transmembrane protein 127 (*TMEM127*), MYC associated factor X (*MAX*), kinesin family member 1B (*KIF1B*), HRas protooncogene (*HRAS*), and ATRX chromatin remodeler (*ATRX*). Dysregulation of kinase signaling pathways, such as PI3K/AKT or mTOR, results in the activation of pathways involved in oncogenic processes (reviewed in (12, 17, 18)).

Focus has now shifted to try and understand the pathogenesis of PPGL and identify similarities in the metabolic changes between PPGLs with different genetic backgrounds. Data indicates that dysregulation of metabolism in both PPGL clusters are linked through the activation of the hypoxia signaling pathway (17). Tumor hypoxia/pseudohypoxia is supposed to have a direct oncogenic or tumor suppressive effect via regulation of various cellular processes. Hypoxia-inducible factors (HIFs) are the key players in cellular responses to hypoxia and the PHD2 – VHL – HIF2 α pathway is critical for erythropoiesis. Partial disturbances in this pathway are associated with erythrocytosis, while substantial changes are involved in tumorigenesis (16), reviewed in (18). Novel *EPAS1/HIF2A* (14, 19) and *EGLN1/2* (16, 20, 21) mutations identified

in PPGLs strongly favor hypoxia signaling as one of the key pathways involved in PPGL development.

The HIF signaling pathway can be triggered by many other oxygen-independent impulses; reviewed in (17). In PPGLs, HIF activation occurs 1) when genes directly involved in regulation of hypoxic responses are mutated (Cluster 1 genes), or 2) when mutations in genes involved in the mTOR and PI3K signaling pathways (Cluster 2 genes) indirectly trigger activation (reviewed in (17)).

Several genes have been implicated in the aggressiveness of PPGLs. Sporadic gene mutations related to chromatin remodeling have been elucidated (22, 23). For instance, *ATRX* gene is involved in telomere maintenance and chromosome integrity (24), as well as in the gene expression and transcriptional repression of non-coding Telomeric Repeat-containing RNA (TERRA) (25). Somatic *ATRX* mutations were identified in 12.6% of PPGLs, and these mutations are associated with alternative lengthening of telomeres and more aggressive tumor behavior (22). Mutation of the histone 3.3 encoding gene (*H3F3A*) was discovered in a patient who presented with recurrent giant cell tumor of the tibia and bilateral pheochromocytomas, and paragangliomas, suggesting that the *H3F3A* mutation was inherited as a driver event in these tumors (23).

PPGLs display a high rate of inherited gene mutations. New data discovered that there are certain genotype-phenotype correlations, including a high risk of metastatic disease development. Therefore, genetic testing should be considered in all PPGL patients (26). Early identification of an underlying genetic mutation can help predict the course of disease and decide on the best treatment strategy. Clinical and biochemical phenotypes can serve as a tool for routing genetic testing (**Table 2**).

Metabolomics and pheochromocytoma

PPGL susceptibility gene mutations result in the dysregulation of cellular key metabolic pathways and subsequent tumorigenic transformation. A metabolic change in transformed cells promotes increased biosynthesis of macromolecules needed for tumor growth and shifts cell metabolism from oxidative phosphorylation to aerobic glycolysis (27).

Measurements of the Krebs cycle and other metabolites in PPGLs confirmed the role of succinate and fumarate in tumorigenesis (**Figure 1**) (28-31). At high levels, both succinate and fumarate act as competitive inhibitors of α -KG-dependent dioxygenases, including HIF prolyl hydroxylases (PHDs), needed for HIF- α proteasomal degradation; reviewed in (17). Hence, high levels of succinate and fumarate lead to the activation of the hypoxia signaling pathway and all metabolic consequences, including activation of protumorigenic mechanisms (27, 32).

SDHx mutations in PPGLs result in the accumulation of succinate, increased levels of methionine, glutamine, and myoinositol, as well as decreased levels of fumarate, glutamate, citrate, isocitrate, and several other metabolites (**Figures 1 & 2**) (30, 31). A high succinate-to-fumarate ratio helps discriminate between PPGLs with and without *SDHx* mutations (29, 30). Moreover, simultaneous measurement of all mentioned metabolites discriminates *SDHx* from non-*SDHx* tumors, with a 100% sensitivity and specificity. This is important for patients with *SDHx*-related tumors who present with low catecholamine secretion or biochemically silent disease. *SDHx* tumors (particularly *SDHB*) display more aggressive behavior and have a high metastatic potential (33).

Accumulation of fumarate in *FH*- and *MDH2*-mutated PPGLs promotes oncogenesis through the succination of proteins, an irreversible covalent bond of fumarate to cysteine

residues in proteins (34-36). Additionally, succinate/fumarate accumulation results in hypermethylated phenotype in *SDHx*- and *FH*-mutated tumors due to inhibition of ten-eleven-translocation methylcytosine dioxygenase (TET) (37, 38). These findings emphasize the interconnection between the Krebs cycle and epigenomic changes (27).

Decreased levels of Krebs cycle intermediates, specifically citrate, isocitrate, and cis-aconitate, detected in *SDHx*- and *VHL*-related PPGLs are also associated with epigenetic and metabolic changes participating in oncogenic processes. Citrate can switch between metabolic pathways and is also used for synthesis of pyruvate, fatty acids, and sterols. Decreased citrate levels has been linked to the induction of epithelial-to-mesenchymal transition, and thus, cancer cell invasion and metastasis (39).

Metabolomic studies conducted on PPGLs revealed other interesting findings. *SDHx*-mutated tumors displayed a lower activity of SDH enzyme (complex II of electron transport chain), but increased activities of complexes I, III, and IV of the electron transport chain. However, the increased activities of the remaining complexes did not lead to full restoration of normal function (40). High levels of glutamine, the second most abundant nutrient in cancer cells, in *SDHx*-related PPGLs suggest that glutamine metabolism is involved in the pathogenesis of these tumors (31). The differences in catecholamine synthesis and secretion among PPGLs with different genetic backgrounds can also be explained based on the metabolic changes resulting from particular mutation, specifically by mutation-dependent changes in energy metabolism (40). In PPGLs, ascorbate, a cofactor in the conversion of dopamine to norepinephrine, is accumulated. Levels of ascorbate were found to correlate with concentrations of catecholamines (31). Perhaps interesting, is the finding that ATP production is not impaired in

SDH deficient PPGLs, implying that Krebs cycle and oxidative phosphorylation function together with aerobic glycolysis in these tumors (27, 31).

Based on the available data, PPGLs are very heterogeneous tumors and exhibit diverse metabolic changes and specific metabolic signatures. Further understanding the PPGL metabolism will help us to improve diagnostic and therapeutic methods.

Developments in the Biochemical Evaluation of PPGL

Upon clinical suspicion of PPGL, a biochemical evaluation using plasma and urinary catecholamines and their O-methylated metabolites, metanephrines should be initiated to confirm diagnosis (3, 33). Proper adherence to suggested guidelines is paramount in collecting evidence of inappropriate catecholamine production (41). Despite recent debate, numerous studies have confirmed that lying supine at rest for 20 minutes prior to blood collection prevents false-positive results due to a postural-related stimulus of norepinephrine secretion (26, 42, 43). Interference is also seen with tricyclic antidepressants, phenoxybenzamine, cigarette smoking, caffeine intake, and consumption of catecholamine-rich foods such as fruits, nuts, cheese, and alcohol (3, 44, 45). Discontinuing the above medications and substances 24 hours prior to blood draw will help increase the diagnostic sensitivity and limit inappropriate disease exclusion due to false-negative test results.

Eisenhofer et al. recently revealed that an optimized curvilinear model with age-adjusted cut-offs for the upper reference limit of normetanephrine improved diagnostic performance with a significant increase in specificity (93.4%), and a non-significant loss in sensitivity (93.7%), compared to the 97.5 percentiles of the reference population, 88.3% and 93.9%, respectively (43). While positive correlations between metanephrine concentration and age were likewise discovered, the upper reference limits only deviated 30% across various age groups. However,

with a single adjustment of the upper reference limit of metanephrine from 0.325 nmol/L to 0.446 nmol/L, the diagnostic specificity increased from 88.3% to 96.0% (43).

The latest reports suggest that age is not the only significant factor requiring reference range adjustment for early and accurate tumor identification. After a unilateral or bilateral adrenalectomy, epinephrine is expected to decrease, which will in turn decrease the plasma concentration of metanephrine (46). Osinga et al. discovered that epinephrine *does* decrease following adrenalectomies, but norepinephrine increases. Thus, an upward adjustment is required for the reference value of norepinephrine, while a downward adjustment is necessary for the metanephrine reference intervals in order to properly detect recurrent disease and prevent unnecessary exposure to radiation with anatomical or functional imaging (46).

In addition to plasma and urinary catecholamines and metanephrines, 3-methoxytyramine, the O-methylated dopamine metabolite, can aid in the identification of exclusively dopamine secreting tumors, which often occur with an asymptomatic clinical presentation (47). Seventy-two percent of patients with *SDHB* mutations and 67% of patients with an *SDHD* mutation had elevated 3-methoxytyramine compared to 39% for *NFI* and *MEN2*, and just 17% for *VHL*-associated tumors (48). Furthermore, in 2012, Eisenhofer et al. reported that 3-methoxytyramine was also significantly elevated in patients with metastases even in the absence of an *SDHB* mutation, and also in patients with metastatic disease secondary to adrenal tumors (33). Therefore, along with previously identified metastatic risk factors such as the presence of an *SDHB* mutation, a tumor diameter greater than 5 cm, and extra-adrenal lesion locations, 3-methoxytyramine is considered a novel biomarker in indicating the likelihood of malignancy (33).

In most countries, laboratories may be unable to measure plasma and urinary catecholamines, metanephrines, and 3-methoxytyramine. Specialized research centers may be required to perform the most complete and accurate biochemical testing until widespread availability is achieved. Institutional differences in biochemical reference ranges and techniques is important to note, indicating that direct laboratory comparisons from various centers should be avoided.

Advances in Functional Imaging

After PPGL is biochemically proven, anatomical imaging with computed tomography (CT) or magnetic resonance imaging (MRI) is performed. Upon CT or MRI lesion confirmation, the patient's biochemical phenotype and tumor size, as well as metastatic potential, plays a determining role in whether further imaging with functional studies is required (49, 50). PPGL patients with a single, epinephrine or metanephrine secreting adrenal mass less than 5 cm, will most likely not benefit from functional imaging, since these tumors are confined to the adrenal gland and present with a small likelihood of metastases (49). However, for lesions greater than 5 cm, secreting norepinephrine or normetanephrine, located extra-adrenally, or associated with a hereditary tumor syndrome such as *SDHB*, the metastatic potential is much higher and functional imaging is necessary to determine the extent of disease (49, 50).

PPGLs overexpress somatostatin receptors (SSTRs) (51-54), which has shifted focus to newly developed imaging modalities targeting SSTRs. DOTA peptides, particularly ⁶⁸Ga-DOTA(0)-Tyr(3)-octreotate (⁶⁸Ga-DOTATATE), are quickly emerging as the gold standard of PPGL functional imaging. While ¹⁸F-FDOPA PET/CT was previously found to be the most sensitive radiopharmaceutical in the detection of metastatic PPGL (55-57), ⁶⁸Ga-DOTATATE PET/CT showed a significantly superior lesion detection rate over other functional and

anatomical imaging modalities, as well as demonstrated a 100-fold higher affinity for SSTR2 than the previous first line SSTR scintigraphy ^{111}In -DTPA-octreotide (Octreoscan) (58). Tan et al. found that utilizing different radiotracers provides imaging of real disease extension, but this practice is time-consuming, impractical, and not cost-effective, which lead to their recommendation that ^{68}Ga -DOTATATE PET/CT become standard of care in determining tumor extent and evaluating metastatic disease (59). Furthermore, avid enhancement on ^{68}Ga -DOTATATE PET/CT could indicate the potential for an additional systemic treatment option with peptide receptor radionuclide therapy (PRRT) (58, 60).

While ^{68}Ga -DOTATATE PET/CT surpassed ^{18}F -FDOPA PET/CT as the functional imaging modality of choice, particularly in sporadic and *SDHB*-related metastatic disease, as well as in head and neck paragangliomas (HNPGLs) (58, 61-63), Darr et al. recently revealed the superiority of ^{18}F -FDOPA PET/CT in detecting multifocality/metastases in somatic *HIF2A* gain-of-function mutation patients (64).

In a recent report from Archier et al., ^{68}Ga -DOTATATE PET/CT failed to detect an *SDHx*-related, small pheochromocytoma in the adrenal gland (61), which again brings attention to the high physiological ^{68}Ga -DOTATATE uptake by healthy adrenal glands (62). In contrast, ^{18}F -FDOPA PET/CT has minimal physiological uptake in the adrenals, and might be beneficial for patients with small, hereditary pheochromocytomas routinely associated with *MEN2* and *NFI* (61).

While the aforementioned functional imaging modalities, particularly ^{68}Ga -DOTATATE PET/CT, are currently unmatched in detection of metastatic disease, their availability is limited (**Figure 3**). On the other hand, ^{18}F -FDG PET/CTs are widely accessible and are the preferred imaging modality for patients with metastatic disease compared to ^{131}I -MIBG, with sensitivities

of 80% and 49%, respectively (26, 65-67). In *SDHx* and *VHL*-related lesions, ^{18}F -FDG PET/CT showed exceptional metastatic localization (65, 66). Until widespread availability of specific radiotracers like ^{68}Ga -DOTATATE and ^{18}F -FDOPA, ^{18}F -FDG PET/CT still offers an inexpensive, high sensitivity option for patients with metastatic disease.

New Therapeutic Perspectives

Targeted molecular therapies

Identifying the genetic background and metabolic alterations in PPGL patients can facilitate a better understanding of PPGL tumorigenesis and allow us to search for new, tumor specific therapeutic targets. Inhibition or alteration of certain metabolic processes involved in tumorigenesis is a very promising therapeutic approach (27). Moreover, based on the genotype-phenotype correlations and metabolomics profile, a personalized therapy could be designed for each patient.

In the initial stages of tumorigenesis, (pseudo)hypoxia seems to play an important role in PPGL development, resulting in abnormal metabolic activity. Thus, modifying or interrupting signaling pathways involved in hypoxic responses seem to be an attractive treatment target, reviewed in (27). Several compounds targeting the HIF signaling pathway are under evaluation (**Table 3**). Several years ago, a tyrosine kinase inhibitor targeting the VEGF receptors – sunitinib – was introduced (68, 69). However, clinical studies showed contradictory results (70-72). Everolimus, a compound that targets the mTOR pathway, has been used in a limited number of PPGL cases, with unsatisfactory results (73, 74). Nonetheless, co-administration of these compounds with other therapeutic agents may show anticancer effects. For example, combined

treatment with NVP-BEZ235, a dual PI3K/mTORC1/2 inhibitor, and lovastatin, a drug known to reduce ERK signaling, showed a significant additive effect, which supports the use of combination therapies in overcoming compensatory upregulation of other pathways and increasing treatment efficacy (75).

A small molecule inhibitor of HIF-2 α (PT2385) is currently being investigated in a Phase 1 clinical trial for the treatment of advanced or metastatic clear cell renal cell carcinoma (ccRCC). Preclinical data indicates that orally bioavailable PT2385 disrupts HIF-2 α activity in ccRCC and thereby blocks the expression of multiple tumorigenic factors responsible for unrestrained cancer cell growth and proliferation, tumor angiogenesis, and suppression of anti-tumor immune responses characteristic of ccRCC. (Peloton Therapeutics Inc., Clinical Trial NCT02293980).

Topoisomerase 1 inhibitors have the ability to interfere with mechanisms that maintain DNA integrity during transcription in both quiescent and dividing cells. *In vitro* studies showed the effectivity of camptothecin, a prototypical topoisomerase 1 inhibitor, in non-dividing PPGL cells. A co-administration of sub-toxic concentrations of 5-azacytidine, a DNA methylation inhibitor, have shown to increase the efficacy of low concentrations of camptothecin (76). Schovanek et al. demonstrated the effectivity of another topoisomerase 1 inhibitor LMP-400 in the preclinical mouse model of metastatic PPGL. LMP-400 was shown to decrease HIF-1 α levels in tumor cells and reduced tumor growth and metastatic potential. Combination therapy with CVD tested *in vitro* showed synergistic effect of LMP-400 and CVD (77).

Restoring the enzymatic activity of nonfunctioning Krebs cycle enzymes, replenishing depleted substrates for the cycle, or inhibiting activity of overexpressed enzymes represent other

possible therapeutic schemes. Small-molecule inhibitors of certain proteins or drugs can restore the functionality of mitochondrial enzymes (**Table 3**), reviewed in (27, 78).

Different therapeutic approaches focus on altering glucose and/or glutamine uptake and metabolism, since these compounds serve as a primary source of energy for cancer cells. Metabolism of glucose and glutamine can be altered by inhibition of uptake (inhibitors of glucose or glutamine transporters), or by inhibition of glycolytic enzymes or glutaminolysis (**Table 3**), reviewed in (27).

Inhibitors of other metabolic pathways, such as lipids and fatty acid synthesis, are also under development (**Table 3**). Recently, inhibitors of fatty acid synthase were demonstrated to selectively target cancer cells for apoptosis (79).

In PPGLs, DNA methylation is also affected by metabolic changes driven by underlying gene mutations. *SDHx*- and *FH*- mutated PPGLs display a hypermethylator phenotype (37), indicating the use of DNA demethylating agents in therapy of some PPGLs (80).

Radionuclide therapy

Surgical resection remains the best treatment option for PPGL. However, an operative approach is not always viable due to extensive metastatic tumor burden. Therefore, inoperable lesions have been treated with systemic targeted radiotherapies, mainly ^{131}I -MIBG (81, 82). Lack of uptake on ^{123}I -MIBG scintigraphy excludes many patients from therapy, driving researchers to discover alternative treatment options for non-surgical candidates. SSTR overexpression in PPGL led to their use as therapeutic targets. By attaching radionuclides (^{177}Lu , ^{90}Y , ^{111}In) via a linker and vector mechanism to a targeting moiety (DOTATATE, DOTANOC, DOTATOC), PRRT has been studied in well-differentiated, advanced neuroendocrine tumors

(NETs)(83-87). While overall results have varied, the majority of studies report the feasibility, effectiveness, and efficacy of PRRT in NETs (84, 87), with increased progression free survival (PFS), as well as patient reported pain relief, symptom regression, and increased quality of life (83). The NETTER-1 Phase III trial compared ^{177}Lu -DOTATATE treatment with the cold analog Octreotide LAR, and found that the median PFS was not reached in the ^{177}Lu -DOTATATE arm, but was 8.4 months for Octreotide LAR – while maintaining a safety profile unremarkable for significant findings related to hematological, renal, or hepatic parameters (87).

Studies are limited regarding PRRT and PPGL, exclusively. Recently, Pinato et al. revealed an overall radiologic disease control rate of 80% in a small cohort of *SDHB*-related metastatic PPGL patients treated with ^{177}Lu -DOTATATE (85). With only one case of significant toxicity and a median time to progression unreached in their 13 month follow-up timeframe, they concluded that PRRT is a viable, tolerable therapy for PPGL. In 2012, a similar study looked at 4 female *SDHD*-related non-metastatic HNPGL patients undergoing ^{177}Lu -DOTATATE treatment, and also reported limited, predictable side effects, with all patients either partially responding or with stable disease (86). Large-scale studies are needed to confirm the data from these limited patient populations, particularly in metastatic sporadic patients without an underlying genetic mutation.

Conclusions

We present the most recent advances in genetics, epigenetics, metabolomics, biochemical, and imaging diagnoses of this rare tumor in order to properly assess disease, decide treatment options, and manage follow-up. At initial presentation, a complete history and physical examination should be performed, with particular attention paid to possible syndromic features.

Urinary and/or plasma catecholamine biochemistry should follow, along with 3-methoxytyramine where available. Blood samples should be drawn with the patient lying supine for at least 20 minutes prior to extraction. Following elevated biochemistry, the next step is anatomical imaging with CT or MRI. Upon lesion confirmation, functional imaging may be appropriate. ^{68}Ga -DOTATATE PET/CT and ^{18}F -FDG PET/CT are the first nuclear scans that should be utilized when metastatic disease is suspected, particularly in *SDHB*-related and HNPGLs. Surgical removal of localized lesions remains the best treatment option for patients with PPGL. When a curative operation is not feasible, targeted molecular therapy or radiotherapy with ^{131}I -MIBG or PRRT should be evaluated. The most effective treatment and follow-up plans are developed with a collaborative, multidisciplinary approach to ensure the patient is adequately assessed from the endocrine, cardiology, oncology, and surgical points of view.

Declaration of Interests

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

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Author contribution statement

The authors of this manuscript contributed equally to its creation.

FIGURE LEGENDS

Figure 1. Metabolic changes and tumorigenesis in PPGL. Schematic representation of mitochondrial genes involved in PHEO development with emphasis to the Krebs cycle enzymes and candidate genes (red) (See Fig.2) and possible areas for therapeutic targets (see Table 3). Dotted lines result from mutations of genes or proteins (red). *SDHB* has been shown to have the abnormal metabolic characteristics. Abbreviations: α -KGDH, alpha-ketoglutarate dehydrogenase; ACO, aconitase; CoA, coenzyme A; COMT, catechol-*O*-methyl transferase; CS, citrate synthase, c-MYC, MYC proto oncogene; eIF-4E, eukaryotic initiation factor 4E; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; FH, fumarate hydroxylase; HIF- α , hypoxia-inducible factor alpha; IDH, isocitrate dehydrogenase; MDH2, malate dehydrogenase; mTORC1, mammalian target of rapamycin complex 1; PDH, pyruvate dehydrogenase; PHD, prolyl hydroxylase domain protein; pVHL, von Hippel-Lindau protein; Raptor, regulatory associated protein of mTOR; RAS, rat sarcoma oncogene; RET, rearranged during transfection proto-oncogene; ROS, reactive oxygen species; S6K, S6 kinase; SDH, succinate dehydrogenase; SUCLG, succinyl-CoA synthase; TMEM127, transmembrane protein 127 (partially adapted from (27, 31).

Figure 2. Whole tumoral metabolome (A) and three-component OPLS-DA model (B) in *SDHx* versus sporadic PHEOs. Note that *SDHx* tumors have higher succinate, taurine and myoinositol metabolic fingerprint. Abbreviations: OPLS-DA, orthogonal partial least square discriminant analysis; GSH, glutathione; Ad; adenine; NAD, nicotinamide-adenine dinucleotide (28) .

Figure 3. A Comparison of Functional Imaging Modalities

Table 1 Genetic syndromes associated with PPGL development

Gene	Locus	Syndrome	Inheritance
<i>SDHA</i>	5p15	Paranganglioma syndrome 5	n/a
<i>SDHB</i>	1p36.13	Paranganglioma syndrome 4	AD
<i>SDHC</i>	1q23.3	Paranganglioma syndrome 3	AD
<i>SDHD</i>	11q23	Paranganglioma syndrome 1	AD, paternal
<i>SDHAF2</i>	11q12	Paranganglioma syndrome 2	AD, paternal
<i>FH</i>	11q42.1	FH	n/a
<i>EPAS1/HIF2A</i>	2p21	Pacak-Zhuang/Paranganglioma-Polycythemia syndrome	n/a
<i>VHL</i>	3p25.3	Von Hippel-Lindau syndrome	AD
<i>NF1</i>	17q11.2	Neurofibromatosis type 1	AD
<i>RET</i>	10q11.21	Multiple endocrine neoplasia type 2	AD
<i>TMEM127</i>	2q11.2	TMEM127	AD
<i>MAX</i>	14q23.3	MAX	AD, paternal

Abbreviations: AD, autosomal dominant; *EPAS1/HIF2A*, endothelial PAS domain protein 1/hypoxia-inducible factor 2 alpha; *FH*, fumarate hydratase; *MAX*, myc-associated factor X; *NF1*, neurofibromin; *RET*, ret proto-oncogene; *SDHA*, *-B*, *-C*, *-D*, succinate dehydrogenase subunits A, B, C, D; *SDHAF2*; succinate dehydrogenase complex assembly factor 2; *TMEM127*, transmembrane protein 127; *VHL*, von Hippel-Lindau gene

Table 2 Genetic testing based on biochemical and clinical phenotype

Biochemical profile	Clinical presentation	Gene mutation
	Family history/syndromic presentation	Test for specific mutation (<i>RET</i> , <i>VHL</i> , <i>NF1</i> , <i>SDHx</i> , <i>MAX</i> , <i>TMEM127</i> , <i>EPAS1/HIF2A</i> , <i>EGLN1/2</i>)
Adrenergic (↑ MN)		<i>NF1</i> , <i>RET</i> , <i>MAX</i> , <i>TMEM127</i>
Noradrenergic (↑ NMN)	Adrenal	<i>VHL</i> , <i>SDHB</i> , <i>SDHD</i> , <i>FH</i>
	Extra-adrenal	<i>SDHB</i> , <i>SDHD</i> , <i>VHL</i>
	Metastatic	<i>SDHB</i> , <i>SDHD</i> , <i>VHL</i> , <i>FH</i>
	Head and neck	<i>SDHD</i> , <i>SDHB</i> , <i>SDHC</i> , <i>VHL</i>
Dopaminergic (↑ MTY)	Adrenal	<i>SDHB</i> , <i>SDHD</i> , <i>VHL</i>
	Extra-adrenal	<i>SDHB</i> , <i>SDHD</i> , <i>VHL</i>
	Metastatic	<i>SDHB</i> , <i>SDHD</i>
	Head and neck	<i>SDHD</i> , <i>SDHB</i> , <i>SDHC</i>
Non-secreting		<i>SDHB</i> , <i>SDHC</i> , <i>SDHD</i>

If there is a family history of PPGL and a known PPGL susceptibility mutation, this mutation should be tested first. Patients presenting with a characteristics of PPGL associated syndromes (listed in Table 1), should be tested for genetic mutations associated with those syndromes. Diagnosis of NF1 is usually made based on the clinical presentation of the disease and no genetic testing is needed. In patients without the family history of PPGL, genetic testing is routed based on the biochemical profile and tumor presentation (localization). For some of the genetic mutations, biochemical profile and tumor presentation are not yet known (1, 27, 88-90).

Abbreviations: *EGLN1/2*, egl-9 family hypoxia inducible factor 1/2; *EPAS1/HIF2A*, endothelial PAS domain protein 1/hypoxia-inducible factor 2 alpha; *FH*, fumarate hydratase; *MAX*, myc-associated factor X; MN, metanephrine; MTY, 3-methoxytyramine; NMN, normetanephrine; *NF1*, neurofibromin; *RET*, ret proto-oncogene; *SDHx*, succinate dehydrogenase subunits; *SDHB*,

C, D, succinate dehydrogenase subunits B, C, D; *TMEM127*, transmembrane protein 127; *VHL*, von Hippel-Lindau gene

Table 3 Potential Molecular/Metabolic Therapeutic Targets in Pheochromocytoma

Therapeutic target	Treatment effects	Examples of agents
HIF signaling		
HIF- α mRNA/protein expression	Inhibition of HIF- α mRNA or protein expression resulting in decreased HIF- α accumulation and activation	Wortmannin, LY94002, GFC-0941, PI-103, Rapamycin, PP242, Aminoflavone, Glyceollins, Topotecan, EZN-2968, 2ME2, ENMD-1198, Geldanamycin and analogues, Vorinostat, YC-1, PX-478, PX-12, cardiac glycosides, FM19G11, HIF-2 α translational inhibitors
HIF- α dimerization	Inhibition of HIF- α /HIF-1 β dimerization	Acridavine, PT2385
HIF binding to DNA	Inhibition of HIF dimers binding to DNA	Echinomycin, polyamides
HIF transcriptional activity	Inhibition of transcription of HIF target genes	Chetomin, Bortezomib, Amphotericin B, Triptolide, AJM290, AW464
Hypoxia	Apoptosis of hypoxic cell	Hypoxia activated prodrugs: TH-302, EO9, AQ4N, PR-104, Tirapazamine, SN30000, TH-4000
Angiogenesis	VEGF, VEGFR inhibition	Sunitinib, Sorafenib, Pazopanib, Bevacizumab
Glycolysis		
Glucose uptake	Inhibition of glucose transport	GLUTs inhibitors: Flavonoids (phloretin, silybin), STF-31, WZB117, ritonavir
HK 1/2	Inhibition	3-Bromopyruvate 2-deoxyglucose, Lonidamine
PDK1	Inhibition (to allow activity of PDH)	dichloroacetate
PFKB3	Inhibition	3PO
LDHA	Inhibition	shRNA, Gossypol/AT-101 and Derivates, Galloflavin
PKM2	Induction of apoptosis	Somatostatin and its derivatives, TLN-232/CAP-232
MCTs	Inhibition of lactate transport	AZD3965 MCT1/2 Specific Inhibitors, CHC, phenylpyruvate, bioflavonoids
Glutaminolysis		

Glutamine uptake	Glutamine transporters inhibition	BCH, GPNA, benzylserine
glutaminase	Inhibition	BPTES/CB-839, Compound 968
GOT2/GPT2	Inhibition	Aminooxyacetate
GDH1	Inhibition	Purpurin/R162, EGCG
Fatty Acid and Lipid Synthesis		
ACLY	Inhibition	SB-204990
Acyl-CoA synthetase	Inhibition	
Acetyl-CoA carboxylase	Inhibition, induction of apoptosis/autophagy	Soraphen A
Fatty acid synthase	Inhibition, induction of apoptosis	Cerulenin, C75, flavonoids
Choline kinase	Inhibition	MN58b
Phospholipid metabolism	Inhibition	metformin
Dysfunctional Krebs Cycle Enzymes And Metabolites		
IDH1/2	Inhibition of IDH mutants, inhibition of 2HG production	Mutant IDH inhibitors
α -ketoglutarate dependent dioxygenases	Restoring the function	α -ketoglutarate analogs
Low citrate	Increase in citrate levels, inhibition of PFK, arrest of glycolysis, induction of apoptosis	Citrate
Other		
DNA methylation	Inhibition of methylation	DNA demethylating agents: decitabine
AMPK	Activation	Biguanides, thiazolidinediones, bevacizumab, erlotinib
LAT1	Inhibition of amino acid transport	2-aminocyclo (2.2.1)-heptane 2-carboxylic acid
SIRT1	Stimulation of SIRT1-dependent deacetylation PGC1 α	Resveratrol
ROS	Neutralizing ROS by antioxidants to reduce HIF- α activation	N-acetylcysteine, vitamin C
ROS	Induction of ROS overproduction	Menandione, Gadolinium texaphyrin, β -lapachone
Antioxidant systems (GSH)	Inhibition to achieve ROS accumulation	Buthionine sulphoximine, Isothiocyanates, Mangafodipir

Adapted from (27)

Abbreviations: 2HG, 2-hydroxyglutarate; 3PO, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-on; Acetyl-CoA, acetyl coenzyme A; ACLY, ATP citrate lyase; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; BCH, 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid; BPTES, bis-2-[5-phenylacetamido-1, 2, 4-thiadiazol-2-yl] ethyl sulfide; CHC, α -cyano-4-hydroxycinnamate; CoA, coenzyme A; DNA, deoxyribonucleic acid; EGCG, epigallocatechin gallate; GDH1, glutamate dehydrogenase 1; GLUT, glucose transporter; GOT2, glutamate oxaloacetate transaminase 2; GPNA, γ -L-glutamyl-p-nitroanilide; GPT2, glutamate pyruvate transaminase 2; HIF, hypoxia-inducible factor; HK, hexokinase; IDH, isocitrate dehydrogenase; LAT1, L-type amino acid transporter 1; LDHA, lactate dehydrogenase A; LY94002, 2-Morpholin-4-yl-8-phenylchromen-4-one; MCT, monocarboxylase transporter; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; mRNA, messenger RNA; PGC1 α , peroxisome proliferator-activated receptor- γ co-activator 1 α ; PHD, prolyl hydroxylase domain-containing protein; PKM2, pyruvate kinase, isoenzyme 2; ROS, reactive oxygen species; SDH, succinate dehydrogenase; shRNA, short hairpin RNA; SIRT1,

sirtuin 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

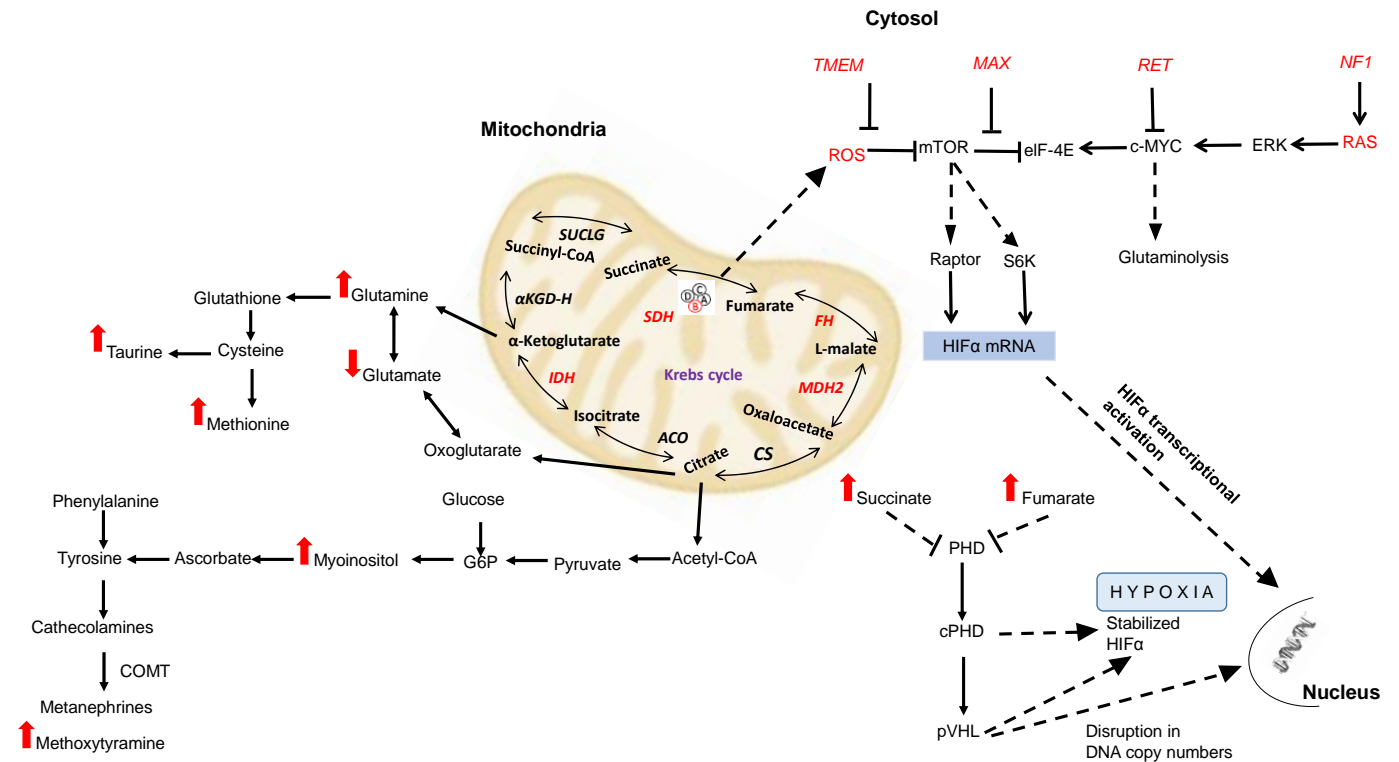


Figure 1

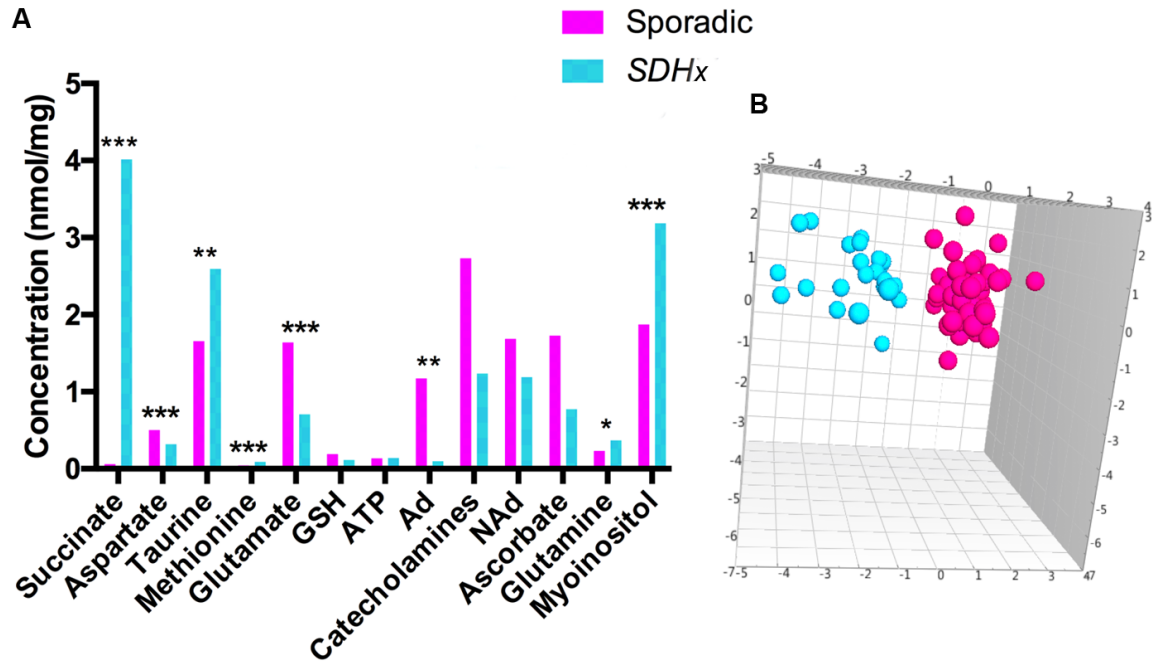


Figure 2

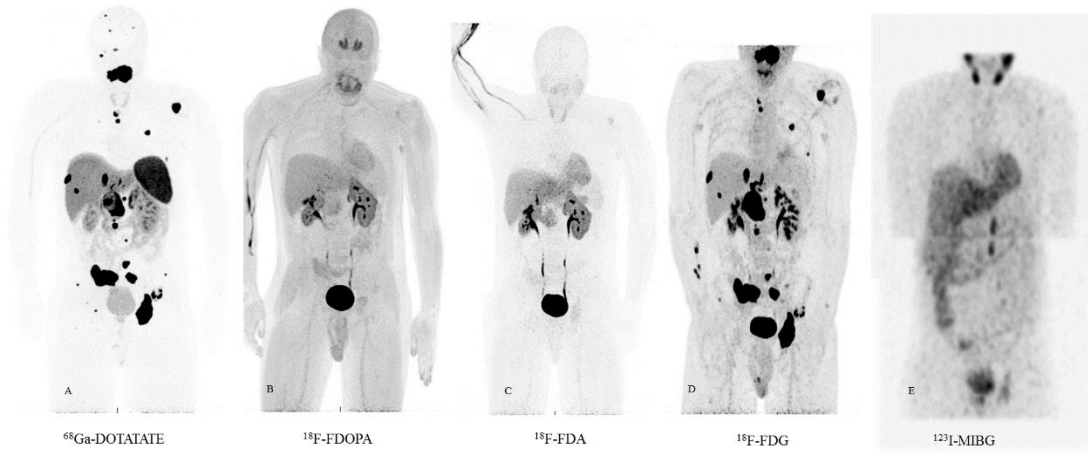


Figure 3

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