The relation between clinical course of pheochromocytoma-paraganglioma (PPGL) and complex genetic etiology

Zależność przebiegu pheochromocytoma-paraganglioma (PPGL) od złożonej etiologii genetycznej

Alicja Szatko*[1], Dorota Leszczyńska[1], Beata Woźniak[1], Piotr Glinicki[1]

[1] *Department of Endocrinology, Centre of Postgraduate Medical Education, Bielanski Hospital, Warsaw, Poland*

KEYWORDS:

- pheochromocytoma
- paraganglioma
- neuroendocrine tumour
- PPGL

ABSTRACT

Recent years brought a remarkable progress in understanding the pathophysiology of rare, neuroendocrine tumors – pheochromocytomas and paragangliomas (PPGLs). Approximately 70% PPGLs are associated with germline or somatic mutation in one of more than 25 identified susceptibility genes, which were grouped into three clusters.

Cluster 1 includes genes encoding proteins involved in Krebs cycle and hypoxia pathway – cluster 1 related PPGL are usually located extra-adrenally (often manifest as head and neck paragangliomas), they are also characterized by noradrenergic/dopaminergic biochemical phenotype and increased metastatic risk. Cluster 2 encompasses genes involved in kinase signaling pathway, PPGL related to cluster 2 are predominantly pheochromocytomas with adrenergic biochemical phenotype, the patients rarely develop metastases. The least known cluster 3, associated with deregulation of Wnt signaling pathway, includes exclusively somatic mutations leading to aggressive pheochromocytomas.

This review summarizes molecular background and the differences between PPGL clusters regarding clinical course, biochemical phenotype, optimal imaging modalities and targeted molecular therapies for metastatic PPGL.

SŁOWA KLUCZOWE:

- guz chromochłonny
- przyzwojak
- guz neuroendokrynny
- PPGL

STRESZCZENIE

Ostatnie lata przyniosły znaczny postęp w poznaniu patofizjologii rzadkich guzów neuroendokrynnych – guzów chromochłonnych i przyzwojaków [pheochromocytomas and paragangliomas (PPGLs)]. Około 70% PPGL związanych jest z mutacjami: germinalną lub somatyczną w jednym z ponad 25 poznanych genów związanych z rozwojem PPGL. W oparciu o rolę kodowanych białek, geny te zostały podzielone na trzy klastry.

Klaster 1 obejmuje geny kodujące białka o aktywności enzymatycznej biorące udział w cyklu Krebsa oraz białka związane ze szlakiem hipoksji. PPGLs związane z mutacjami genów należących do klastra 1 zazwyczaj rozwijają się z autonomicznych zwojów współczulnych lub przywspółczulnych (przyzwojaki głowy i szyi), charakteryzują się zwiększoną sekrecją metabolitów noradrenaliny i/lub dopaminy oraz znacznym ryzykiem tworzenia przerzutów. Klaster 2 to geny kodujące białka związane ze szlakiem sygnałowym kinaz – u chorych z PPGL uwarunkowanymi mutacjami należącymi do tego klastra zazwyczaj stwierdza się guzy chromochłonne, podwyższone stężenie metabolitów adrenaliny w diagnostyce biochemicznej, a rozsiana postać występuje rzadko. Najmniej poznany klaster 3 skupia geny związane ze szlakiem sygnałowym Wnt – wśród tych pacjentów dominują guzy zlokalizowane w nadnerczach o agresywnym przebiegu.

W tym artykule poglądowym zostały przedstawione uwarunkowania molekularne podziału PPGLs na klastry, a także kliniczne, biochemiczne, obrazowe oraz terapeutyczne implikacje proponowanej klasyfikacji.

Introduction

Pheochromocytomas and paragangliomas (PPGLs) belong to heterogenous neuroendocrine tumors producing catecholamines. In the World Health Organisation (WHO) classification of endocrine and neuroendocrine tumors from 2022, PPGL were unified as "paragangliomas", while pheochromocytomas are referred as "intra-adrenal paragangliomas" (1).

PPGL present the highest degree of heritability among all solid tumors (2). Up to 35% of PPGL patients harbour germline mutation, while in additional 35-40% of PPGL patients somatic mutations can be found (2-6). Thus, in more than 70% of affected patients, the development of PPGL can be attributed to pathogenic mutation in one of more than 25 identified germline or somatic genes (2-7). Among PPGL susceptibility genes, 12 the most common driver genes are identified in majority of patients (7).

Recent integration of the multi-omics research led to better understanding of PPGL pathogenesis and enable to group of driver mutations into three clusters referring to the deregulated molecular pathway: pseudohypoxia (cluster 1), kinase-signaling (cluster 2), and Wnt signaling (cluster 3) (2, 3). The genetic testing has been recommended in every patient diagnosed with PPGL (optimally using time-effective Next Generation Sequencing (NGS) of the tumor tissue) and it was proven to positively influence the management and outcome (8, 9). Furthermore, the implementation of PPGL clusters allows to determine biochemical phenotype, clinical presentation, optimize diagnostics and treatment of metastatic disease, which can be considered a milestone in personalized management of PPGL (2, 3, 7).

In this review, recent insights regarding PPGL genetics, and its clinical implications are summarized.

Molecular background of PPGL clusters

Pseudohypoxia cluster 1

The first cluster of the novel molecular classification of PPGL comprises two subclusters: 1A: Krebs-cycle associated genes and 1B: hypoxia-signaling pathway-related genes (2, 3). The former subcluster includes germline succinate dehydrogenase subunits (SDHx) A, B, C and D (SDHA, SDHB, SDHC and SDHD), succinate dehydrogenase complex assembly factor 2 (SDHAF2), malate dehydrogenase 2 (MDH2), fumarate hydratase (FH), mitochondrial glutamic-oxaloacetate transaminase (GOT2), dihydrolipoamide S-succinyltransferase (DLST), 2-oxoglutarate-malate carrier (SLC25A11), and somatic isocitrate dehydrogenase 1 (IDH1) gene mutations (2, 3). The subcluster 1B encompasses von Hippel-Lindau (VHL) tumour suppressor, Egl-9 prolyl hydroxylase-1 and -2 (EGLN1/2 encoding PHD1/2), hypoxia-inducible factor 2α (HIF2A/EPAS1), and iron regulatory protein 1 (IRP1) genes (2, 3).

The mutations of the Krebs-cycle associated genes (cluster 1A) lead to impaired electron chain transport and oxidative phosphorylation in the mitochondria, which forces the switch to aerobic glycolysis (known as the Warburg effect) to generate adenosine triphosphate (ATP) (3, 10-12). The disturbance in Krebs-cycle results in accumulation of oncometabolites, namely fumarate, succinate, or 2-hydroxyglutarate (3). The consequence of increased concentration of oncometabolites (mainly succinate and fumarate) leads to DNA hypermethylation, suppression of prolyl hydroxylase,

decreased hypoxia-inducible factor α (HIF-α) hydroxylation, ubiquitination, and proteasome-mediated degradation (3, 13, 14). Aerobic glycolysis may further contribute to tumor cell proliferation via acidification of microenvironment, production of reactive oxygen species (ROS), and modulation of immune response (11).

The influence of HIF-α connects cluster 1A and cluster 1B. Subunits of HIF-α have very short half-life under sufficient oxygen supply (15). Although, decreased oxygen saturation leads to diminished HIF-α hydroxylation and increased stability (15). The cluster 1 was named "pseudohypoxia" in reference to the initially described pathway activation by decreased oxygen concentration, while prefix "pseudo" was added to mark other factors than lower oxygen saturation contributing to the pathway activation (16).

The catalogue of cluster 1B susceptibility genes starts with mutations (predominantly germline, but also somatic) in VHL gene, encoding von Hippel-Lindau suppressor protein which belongs to E3 ubiquitin ligase complex, and recognizes HIF heterodimer (HIF-α and HIF-β) and delivers it to the proteasome for degradation (17). However, to be identified by Hippel-Lindau suppressor protein, HIF-1α and HIF-2α need to be hydroxylated by propyl hydroxylase domain proteins (PDH1–3) – loss-of-function germline mutations in genes encoding isoforms 1 and 2 of PHD belong to cluster 1B (3, 17). Also gain-of-function somatic HIF2A/EPAS1 mutations prolong the half-life of HIF and predispose to PPGL (18). Furthermore, there was a reported case of PPGL-polycythaemia syndrome associated with IRP1 frameshift mutation, resulting in substantially decreased IRP1 protein level (19). Consequently, lack of translational suppressor (IRP-1 protein) of HIF-2α, resulted in upregulation in hypoxia pathway and erythropoietin (EPO) expression (19).

Kinase-signaling cluster 2

The kinase-signaling cluster includes the majority of PPGL (50-60%), driven predominantly by germline mutations (17). This group encompasses proto-oncogenes (rearranged during transfection (RET) tyrosine kinase gene and H-ras GTPase proto-oncogene (HRAS) and genes encoding transmembrane protein 127 (TMEM127), neurofibromin 1 (NF1) and MYC-associated factor X (MAX) (17). There are also reports including genes encoding fibroblast growth factor receptor 1 (FGFR1), MER proto-oncogene, tyrosine kinase (MERTK), tyrosine-protein kinase Met (MET), nerve growth factor receptor (NGFR) and proto-oncogene B-Raf (BRAF) into cluster 2 (2, 3, 20).

Driver mutations in cluster 2 genes result in the activation of kinase signaling pathways: phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin kinase (mTOR) and RAS-MAPK for RET, NF1, TMEM127 and HRAS, while pathogenic mutations in MAX lead to overstimulation of MYC (17). The consequences of described alterations in kinase signaling pathways include uncontrolled cell growth and proliferation, chromatin remodelling, angiogenesis, and metabolic switch from oxidative phosphorylation to glycolysis and degradation of glutamine (2, 3, 21).

Wnt signaling cluster 3

The last, yet least explored cluster 3 includes only somatic driver mutations in genes regulating Wnt/ β-catenin signaling

pathway (2, 3). This group comprises of mastermind like transcriptional coactivator 3 (*MAML3*) fusion genes i.a. transcription factor 4 (*TCF4*)-*MAML3* and upstream binding transcription factor, RNA polymerase I (*UBTF*)-*MAML3* and cold shock domain-containing E1 (*CSDE1*) gene (2, 3, 4). Gain-of-function mutations of the abovementioned genes in this cluster promote tumor cell growth, proliferation, adhesion, motility, and ability to metastasize (4).

PPGL susceptibility genes associated with DNA hypermethylation

Apart from described three clusters, there have been identified other PPGL susceptibility genes – involved in deoxyribonucleic acid (DNA) hypermethylation, namely genes encoding histone 3.3 (*H3F3A*), DNA methyltransferase (*DNMT3A*) and isoform β of kinesin family member 1B (*KIF1Bβ*) (3, 20, 22, 23, 24). Although the role of *KIF1B* in pathogenesis of neural crest tumors and neuroblastomas was proven in independent studies, the involvement of *KIF1B* in PPGL development remains debatable (25, 26). In the report in 2008 by Yeh et al. family carrying a *KIF1B* missense variant, the mutation was confirmed only in one allele, there was also no evidence of loss of heterozygosity at the somatic level (27). Furthermore, the recent extended molecular analysis of this family revealed that the brother of the proband, who developed bilateral pheochromocytoma at the age of 31, was a carrier of germline *MAX* gene mutation, which was also confirmed in all family members affected by PPGL (28).

Pathogenic mutations in PPGL susceptibility genes associated with DNA hypermethylation result in decreased degradation of HIF-2α and contribute to higher metastatic potential (29). These similarities with pseudohypoxia cluster justify including genes associated with DNA hypermethylation into cluster 1.

Cluster-specific clinical features of PPGL

Better understanding of molecular basis of PPGL provides insight into cluster-specific clinical course, regarding i.a., location of the tumor, risk of metastases and comorbidities. Patients with mutations included in the pseudohypoxia cluster often develop PPGL at younger age, they are also predisposed to multifocal, recurrent PPGL with increased metastatic potential (30). Among all *SDHx* genes, *SDHB* mutations are associated with the highest metastatic risk – up to 75%, thus *SDHB* is considered poor prognostic factor in genetic algorithms (3, 31). The patients with *SDHB* mutations usually develop extra-adrenal tumors located in abdomen, pelvis, or thorax, less often these patients are diagnosed with head and neck paragangliomas (HNPGL) (32). PPGL associated with *SDHD* pathogenic mutations usually present as multiple HNPGL, with low metastatic risk (33). PPGL harbouring *SDHC* mutations are predominantly located in extra-adrenal sites (head and neck), while metastases are diagnosed in 23% of cases (33). *SDHA* mutations were described as a rare cause of abdominal, thoracic, and HNPGLs, with high metastatic potential – two-thirds of affected patients develop metastases (3). Mutations in *SDHAF2* also predispose to extra-adrenal tumors, mainly HNPGL (34). Patients with *FH*- or *MDH2*- related PPGL usually present with multiple tumors and marked risk of metastatic disease (31).

On the contrary to PPGL harbouring *SDHx* mutations, pathogenic mutations in *VHL* are mainly associated with development of pheochromocytomas, multifocal or bilateral in 43% up to 45% of patients, while in less than 5% of affected subjects, metastases are diagnosed (31, 35, 36). Interestingly, mutations in *VHL* are associated with rare hereditary tumor syndrome, but pheochromocytomas are reported only in subtype 2, in 30-50% of patients as the first manifestation of the disease (31). H*IF2A/EPAS1*-related PPGL can occur both in adrenal glands and in extra-adrenal sites (3).

PPGL associated with cluster 2 genes develop nearly exclusively in the adrenals, they are also characterized by low metastatic risk (less than 5%) (37). Syndromic manifestation of germline mutations in *RET* protooncogene includes three clinical subtypes: multiple endocrine neoplasia 2A (MEN2A), multiple endocrine neoplasia 2B (MEN2B) and familial medullary thyroid carcinoma (MTC), depending on the associated pathologies (31). Approximately half of MEN2 patients develop pheochromocytoma (up to 80% bilateral disease), while the PPGL is the first manifestation of MEN2 only in 12-15% of cases (31, 38). PPGL in neurofibromatosis type 1 (von Recklinghausen syndrome, caused by *NF1* mutations) are found in 0.1-5.7% of patients, they are often unilateral pheochromocytomas, up to 10 % of patients develop metastases (31). It is noteworthy that in up to 20% of pheochromocytomas that are considered sporadic, somatic mutations in *NF1* can be identified (39). *MAX* pathogenic mutations usually manifest as pheochromocytomas, often bilateral or multiple (40). Most tumors associated with *TNEM127* mutations also develop in adrenal glands (41).

Somatic mutations of cluster 3 genes manifest predominantly as pheochromocytomas (2). Tumours of this cluster are characterized by aggressive phenotype, high risk of metastases and recurrence (2). In the study of Fishbein et al., PPGL associated with *MAML3* fusion gene showed very high expression of Ki-67, associated with tumour cell growth and proliferation (2).

Summary of mutations associated with multiple clinical manifestations, not only PPGL, was presented in Table 1.

Differences in biochemical phenotypes

In recent years, we witnessed remarkable progress in understanding not only PPGL molecular pathogenesis but also catecholamine biosynthesis, which resulted in the switch from measurement of total catecholamines to plasma-free or fractionated urinary metanephrines in the biochemical diagnosis of PPGL (8). Current consensus of Working Group on Endocrine Hypertension of the European Society of Hypertension published in 2020 recommends the measurement of plasma-free or fractionated urinary metanephrine and normetanephrine, complemented by the determination of 3-methoxytyramine concentration in plasma for detecting dopamine-producing tumours (measurement of 3-methoxytyramine in 24 hr urine collection for PPGL diagnosis is not recommended since the most of dopamine metabolite excreted in urine derives from renal uptake and decarboxylation of circulating L-dopa) (8, 42). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is preferred analytical technique, while 2-fold increase above upper reference limit (>2 URL) provides a high suspicion of a PPGL (3, 8). Importantly, patients tested because of genetic risk have high pretest prevalence of PPGL and measurement of

Table 1. Characteristics of the pheochromocytomas-paragangliomas (PPGLs) susceptibility genes associated with syndromes or other clinical manifestations.

	Gene	Encoded protein	Inheritance	Associated conditions
CLUSTER 1A	SDHA	Succinate dehydrogenase subunit A	AD	GIST, RCC, pituitary NET
	SDHB	Succinate dehydrogenase subunit B	AD	GIST, RCC, pituitary NET, pulmonary chondroma
	SDHC	Succinate dehydrogenase subunit C	AD	GIST, RCC, pituitary NET
	SDHD	Succinate dehydrogenase subunit D	AD, paternal	GIST, RCC, pituitary NET, pulmonary chondroma
	FH	Fumarate hydratase	AD	Cutaneous and uterine leiomyomatosis, HLRCC
	MDH2	Malate dehydrogenase 2	AD	Early-onset severe encephalopathy
CLUSTER 1B	VHL	Von Hippel-Lindau suppressor	AD	CNS hemangioblastoma, RCC, serous pancreatic cystadenoma, pancreatic NET, retinal angioma, endolymphatic sac tumor, papillary epididymal cystadenoma
	EGLN1	Egl-9 prolyl hydroxylase-1	AD	Polycythemia
	EGLN2	Egl-9 prolyl hydroxylase-2	AD	Polycythemia
	HIF1A/EPAS1	Hypoxia-inducible factor 2-alph	Unknown	Polycythemia, somatostatinoma retinal abnormalities
	KIF1B	Kinesin Family Member 1B	AD	Neuroblastoma
CLUSTER 2	RET	Rearranged during transfection (RET) tyrosine kinase	AD	MEN2A: MTC + PPGL + PH MEN2B: MTC + PPGL + marfanoid habitus, ganglioneuromatosis of the gut/oral mucosa, joint laxity, skeletal deformity
	NF1	Neurofibromin 1	AD	Multiple cafe au lait spots, iris hamartoma (Lisch nodule), neurofibroma, optic glioma, inguinal and axillary freckling, breast carcinoma, sarcoma, GIST, melanoma, cognitive defects
	TNEM 127	Transmembrane protein 127	AD	RCC
	MAX	MYC-associated factor X	AD, paternal	Pituitary NET, renal oncocytoma

Abbreviations: AD – autosomal dominant; **GIST** – gastrointestinal stromal tumours; **RCC** – renal cell carcinoma; **NET** – neuroendocrine tumours; **HLRCC** – papillary renal cancer type 2; **CNS** – central nervous system; **MEN2A** – multiple neoplasia type 2A; **MTC** – medullary thyroid carcinoma, **PH** – primary hyperparathyroidism; **MEN2B** – multiple neoplasia type 2B.

plasma-free metanephrines was proved to be optimal for initial biochemical testing in this group (43).

Mutations in cluster 1 genes predominantly lead to development of tumors with noradrenergic or dopaminergic phenotype, in some cases, mild elevation in metanephrine is observed (35, 44). PPGL associated with pseudohypoxic cluster genes are characterized by lower catecholamine content in tumor tissue, probably attributed to continuous release patterns (45). Dopaminergic phenotype is associated with increased risk of metastatic disease (46). Rarely, PPGL caused by mutations in cluster 1 genes remain biochemically silent (no significant elevation in free plasma metanephrines or 3-methoxytyramine is observed) – this phenomenon occurs in HNPGLs and SDHB-associated extra-adrenal lesions (30, 47). Lack of epinephrine secretion is related to the molecular background of cluster 1. Pathogenic mutations of pseudohypoxia cluster lead to decreased degradation of HIF-2α, which has the ability to inhibit the expression of phenylethanolamine N-methyl transferase (PNMT) – an enzyme that transfers a methyl group to norepinephrine to form epinephrine (48, 49).

The secretory profile of PPGL associated with cluster 2 mutations differs significantly from cluster 1. The former tumors are characterized by adrenergic phenotype (except

PPGL clusters			
1 pseudohypoxia		**2 kinase signaling**	**3 Wnt signaling**
1A	**1B**		
SDHA SDHB SDHC SDHD SDHAF2 FH MDH GOT2 DLST SLC25A11 IDH1 **H3F3A**[1] **DNMT3A**[1] **KIF1Bβ**[1]	VHL PDH1-2 HIF2A /EPAS1 IRP1	RET HRAS TMEM127 NF1 MAX FGFR1 MERTK MET NGFR BRAF	CSDE1 MAML3 fusion genes
location extra-adrenal		adrenal	adrenal
age of initial diagnosis early		rather late	unknown
multifocal disease often		rare	unknown
metastatic risk 40.5%	11.0%	4.1%	11.4%
biochemical phenotype noradrenergic /dopaminergic		adrenergic	unknown CgA overexpressed

Figure 1.

The summary of PPGL related genes grouped into three clusters depending on the deregulated pathway. Cluster-specific most common PPGL clinical features including metastatic risk [based on the results of Crona et al. (37)], and biochemical phenotypes are also presented.

Abbreviations: PPGL – pheochromocytoma-paraganglioma; **CgA** – chromogranin A.

[1] Mutations in genes associated with DNA hypermethylation lead to decreased degradation of HIF-2α – these similarities with pseudohypoxia cluster justify including genes associated with DNA hypermethylation into cluster 1.

for mainly noradrenergic *MAX*-related PPGL), high content of catecholamines in tumor tissue, and often episodic release pattern (35, 44, 45). The biochemical phenotype of PPGL related to cluster 3 was not established. Interestingly, in the study of Fishbein et al. these neoplasms showed the highest expression of *CHGA* – gene encoding chromogranin A (CgA) (2).

Cluster-specific PPGL susceptibility genes, clinical features and biochemical phenotypes were summarized in Figure 1.

Optimal imaging modality – one for all or specific for the cluster?

The choice of imaging modality for PPGL assessment is crucial to locate the tumor before the scheduled surgery. When combined with functional imaging, specificity and sensitivity further improve, and it provides also additional advantages if targeted radionuclide therapy is planned (8). The result of molecular analysis revealing pathogenic mutation associated with PPGL may help to choose the individual patient's imaging and functional modalities.

Since the PPGL related to cluster 1A develop mainly in the extra-adrenal sites, magnetic resonance imaging (MRI) is superior to computed tomography (CT) imaging for these patients, especially if HNPGL is suspected. However, there are a few exceptions to this rule – CT imaging is preferable for the assessment of small lesions (due to better spatial resolution and fewer motion artefacts), thoracic structures (e.g., when the lung metastases are suspected) and it allows to better assess PGL extension to the temporal bone (3). Pheochromocytomas (associated with clusters 1B, 2 and 3) present different diverse morphological features on CT imaging: heterogenicity, areas of necrosis, calcifications, haemorrhages, or cystic changes (8). Nevertheless, on unenhanced attenuation CT imaging, nearly all pheochromocytomas are >10 Hounsfield units (HU) which distinguishes them from adenomas (50, 51). On MRI, pheochromocytomas are classically described as hyperintense on T2-weighted imaging (light-bulb sign, present in one-third of the tumors), while typical hypointensity on T1-weighted imaging may be altered by the presence of haemorrhages or fat (3). Since pheochromocytomas usually do not contain a remarkable amount of intracellular lipid, signal loss on out-of-phase chemical shift MRI imaging may be helpful in diagnostic process – in the study of Araujo-Castro et al., signal loss on out-of-phase was observed in 39% of pheochromocytomas and 90% of other lesions (52).

Functional imaging is an integral part of PPGL diagnostics, and it is recommended for initial screening (and optional for the follow-up) of *SDHx* mutation carriers, staging if multiple lesions or metastases are present, before the surgery of pheochromocytomas ≥5 cm, and after the operative treatment of sympathetic paragangliomas, oligometastatic or multifocal disease (3). In case of *SDHx-associated* PPGL gallium-68 DOTA-conjugated

Table 2. Cluster-specific and sporadic pheochromocytoma-paraganglioma biochemical biomarkers and recommended radionuclide imaging techniques, adapted from Taieb et al. (53).

In PPGL associated with mutations of Wnt signaling pathway (cluster 3) increased plasma concentration of metanephrine or normetanephrine may be observed, however further studies of cluster 3 PPGL are needed to establish distinctive biochemical phenotype and optimal radionuclide imaging techniques (54).

	Cluster 1A Krebs-cycle -associated	Cluster 1B hypoxia-signaling pathway-related	Cluster 2 kinase signaling	Sporadic PHEO	Sporadic PGL, multifocal, metastatic PPGL
Biomarker in plasma	NM, 3-MT	NM	M, NM	M, NM	NM, 3-MT
	CgA				
Optimal radionuclide imaging modality	**First choice**				
	68Ga-SSA	18F-FDOPA	18F-FDOPA	18F-FDOPA or 123I-MIBG	68Ga-SSA
	Second choice				
	18F-FDG and 18F-FDOPA	68Ga-SSA or 123I-MIBG	68Ga-SSA or 123I-MIBG	68Ga-SSA	18F-FDG[1] and 18F-FDOPA

Abbreviations: PPGL – pheochromocytoma/paraganglioma; **PHEO** – pheochromocytoma; **PGL** – paraganglioma; **NM** – normetanephrine; **3-MT** – 3-methoxytyramine; **M** – metanephrine; **CgA** – chromogranin A; **68Ga-SSA** – gallium-68 DOTA-conjugated somatostatin receptor-targeting peptide positron-emission tomography-computed tomography; **18F-FDOPA** – 6-[18F]-fluoro-l-3,4-dihydroxyphenylalanine positron-emission tomography-computed tomography; **123I-MIBG** – 123I- meta-iodobenzylguanidine; **18F-FDG** – fluorine-18 fluorodeoxyglucose positron-emission tomography-computed tomography.

[1] Recommended for sympathetic PGL, multifocal or metastatic PPGL.

somatostatin receptor-targeting peptide positron-emission tomography-computed tomography ([68Ga]-DOTA-SSA PET/CT) shows the highest sensitivity since these lesions strongly express the somatostatin receptor 2 (SSTR2) (53). For *VHL* related PPGL, 6-[18F]-fluoro-l-3,4-dihydroxyphenylalanine positron-emission tomography-computed tomography ([18F]F-DOPA PET/CT) is an optimal functional modality, because these tumours are characterized by increased expression of the L-type amino-acid transporter, SSTR2 expression is in comparison to *SDHx* related PPGL less pronounced (3, 53). [18F]F-DOPA PET/CT should be also used for *NF1/RET/MAX* associated pheochromocytomas assessment as preferred modality (53). When the availability of PET is limited, 123/131I- meta-iodobenzylguanidine (MIBG) may be an alternative (53). However, small PPGL lesions (less than 7 mm in size) and tumors associated with *SDHx* mutations can lead to false-negative 123/131I-MIBG results (53).

Plasma biomarkers and optimal radionuclide imaging techniques for cluster-specific and sporadic PPGL were presented in Table 2.

Treatment

Surgical treatment, after alpha-adrenoceptor blockade (if necessary), is the only curative option for PPGL. If the radical surgery is not possible or not preferred (e.g., in case of HNPGL encroaching neighbouring structures), active surveillance, stereotactic radiosurgery, and conventional or targeted radiotherapy should be considered (8).

Approximately 10-20% of PPGL patients develop metastatic disease (55). Patients with metastatic PPGL should be referred to specialized, multidisciplinary centers, while the choice of the therapy should be individualized. Before the treatment, it is recommended to perform staging and assessment of disease progression (by imaging usually over an interval of 3 months) (8). However, patients with multiple PPGL metastases in liver, brain and lungs should be treated immediately (8). In case of rapid progression (<6 months) chemotherapy [cyclophosphamide, vincristine, dacarbazine (CVD)] is a preferred option, and temozolomide is an alternative (3, 8). It is noteworthy that patients with *SDHx* respond better than patients with sporadic PPGL (70% vs. 30%.) to classical chemotherapy (8, 56). Radionuclide therapy [high-specific-activity [I131]MIBG (Azedra)/conventional [I131]MIBG or tetra-azacyclododecanetetra-acetic acid (DOTA) conjugated with tyrosine-containing somatostatin analogue Tyr3-octreotate (TATE) radiolabelled with lutetium-177 (177Lu-DOTATATE, Lutathera) peptide receptor radionuclide therapy (PRRT)] is recommended for patients with slow or moderate progression of PPGL (8). If the disease further progresses, temozolomide, tyrosine kinase inhibitors (TKI, sunitinib or cabozantinib) and immunotherapy (pembrolizumab) should be considered (3).

Apart from the therapies already used in clinical practice, there are numerous clinical trials of new treatment options which target molecular pathways in metastatic PPGL. For cluster 1 associated metastatic PPGL, chemotherapy (CVD, temozolomide with or without poly (ADP-ribose) polymerase (PARP) inhibitors (olaparib), PRRT and HIF-2α inhibitors (belzutifan) either proven their efficacy or are potentially optimal regarding the targeted pathways (3). Patients with metastatic PPGL related to cluster 2 may profit the most from [131I] MIBG therapy, TKI, RAF/MEK/ERK inhibitors or PI3K/AKT/ mTORC1 inhibitors (3). For the cluster 3 metastatic PPGL, potential treatment options are limited. However, antitumor activity of the porcupine (PORCN) inhibitor WNT974 and the β-catenin inhibitor PRI-724 showed efficacy in neuroendocrine tumor cell lines (57).

Conclusions

Novel molecular classification of PPGL susceptibility genes provides valuable information concerning clinical course, biochemical phenotype, and optimal imaging modalities for individual PPGL patients, it also brings us closer to personalized management of PPGL. However, there are still patients diagnosed with PPGL who were not informed about the importance of molecular testing. Molecular analysis (optimally from tumor tissue) and genetic counselling should be considered essential part of PPGL patients management. Since known genetic predisposition may help to effectively navigate through diagnostics, treatment, and follow-up of PPGL tumors, cost-effectiveness of molecular testing cannot be questioned. Further research and in-depth understanding of molecular, but also metabolomic and immune landscape of PPGL may enable an introduction of patient-tailored therapeutic options, especially for metastatic PPGL.

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