Potential Biomarkers of Metastasizing Paragangliomas and Pheochromocytomas

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Paragangliomas and pheochromocytomas (PPGLs) are rare neuroendocrine tumors formed from paraganglionic tissue. Since 2017, PPGLs have been classified as tumors with variable potential to metastasize. Metastasizing PPGLs are usually difficult to diagnose and require evidence of regional or distant metastasis. Data on diagnostic and prognostic molecular markers for PPGL malignancy are limited, and many of the proposed factors remain controversial. There is a significant gap in the understanding of tumor pathogenesis, as well as the treatment and management of patients with PPGLs. This entry summarized the current findings on the potential markers for distinguishing between metastasizing and benign tumors, as well as on the prediction of aggressive behavior of PPGLs, especially of those localized in the head and neck region.

Keywords: paragangliomas and pheochromocytomas ; head and neck paragangliomas ; malignancy ; diagnostic and prognostic markers

1. Definition, Localization, and Distribution

Paraganglia represent groups of paraneurons derived from neural crest cells during embryonic development and are divided into sympathetic and parasympathetic. Sympathetic paraganglia consist of chromaffin cells and are involved in the secretion of catecholamines (norepinephrine, epinephrine, and dopamine), while parasympathetic paraganglia consist of glomus (nonchromaffin) cells and act as chemoreceptors [1]. Sympathetic paraganglia are associated with ganglia of the sympathetic trunk, celiac, renal, adrenal, and hypogastric plexuses. The tumors that arise from the largest sympathetic paraganglia forming the adrenal medulla are called pheochromocytomas (PHEOs). Tumors developing from paraganglia outside the adrenal gland are termed paragangliomas (PGLs). Parasympathetic paraganglia include supracardiac paraganglia, paraganglia of the carotid body, middle ear, and larynx, as well as paraganglia distributed along the vagus nerve and several other smaller paraganglia [2]. These paraganglia are common throughout the body, but most are found in the head and neck area [3]. The most common sites for head and neck paragangliomas (HNPGLs) include the carotid body followed by middle ear and vagal glomus [4]. In rare cases, HNPGLs can develop at other sites of the head and neck, such as nasopharynx, nasal cavities, paranasal sinuses, larynx, thyroid gland, and orbit [5]. Approximately a third of HNPGLs can secrete catecholamines [6]. Catecholamine-secreting PPGLs are termed functional while non-secreting ones are often termed non-functional and predominantly include HNPGLs. The main epidemiological data for PHEOs and PGLs are presented in **Table 1**.

Table 1. Epidemiological data for PPGLs.

Parameter	HNPGLs			PHEO	Other Extra-Adrenal PGLs
	CPGL	MEPGL	VPGL		
Mean age at diagnosis	40–50 * [7][8]	55 [3]	41–47 [3]	40–50 [9]	40–50 [9]
Female/male ratio	2:1–8:1 ** [10][11]	3:1–9:1 [12]	2:1–8:1 [12]	1:1 [9]	1:1 [9]
Multifocal cases, %	10–25 [4]	10–50 [13]	10 *** [14]	8 [15]	33 [15]
Metastatic cases, %	4–6 [12]	2 [12]	16–19 [10][12]	10 [9]	2.5–50 [9]

CPGL, carotid paraganglioma, MEPGL, middle ear paraganglioma, VPGL, vagal paraganglioma. * Metastatic cases are characterized by a 10-year earlier age of diagnosis. ** The ratio is higher in populations living at high altitudes under hypoxic conditions. *** Incidence of multiple paragangliomas increases to 30–40% in patients with a positive family history.

2. Metastatic Disease

2.1. Clinical Characteristics

Overall for PPGLs, patients with metastatic disease are younger than ones with non-metastasizing tumors at the time of diagnosis. The female/male ratio is lower for metastasizing PPGL patients than for PPGL ones. There are no differences in systolic and diastolic blood pressure, heart rate, and body mass index (BMI) between groups [16]. Extra-adrenal location of paragangliomas is more frequently associated with the risk of malignancy in comparison with intra-adrenal sites [17]. Such clinical parameter as multifocality was shown not to play a significant role in the progression of metastasizing PPGLs [15]. Local recurrence and metastasis in non-chromaffin tissues are likely to co-occur [18][19].

Tumor size and weight are often noted as potential independent predictors of aggressiveness in PPGLs. However, the data on the correlation between this tumor characteristic and the risk of malignancy remain controversial. In the study of a representative set of PPGLs, the correlation of larger primary tumor size with rapid disease progression was revealed [15]. However, none of the HNPGLs presented as part of the sample set analyzed developed metastasis. Similar results were obtained in the study of Khadilkar et al. who found a significantly larger primary tumor size in patients with metastasizing PPGLs, but only two metastatic HNPGL cases were included in the analysis [20]. Additionally, in several studies of PHEOs and PGLs, metastasizing tumors exhibited larger tumor size and/or weight than benign ones [21][22][23]. In contrast, Thompson showed no statistically significant differences in increased tumor size alone and malignancy that was also confirmed in a more recent study of patients with metastasizing PPGLs [24][25].

2.2. Biochemical Markers

Most sympathetic paragangliomas produce catecholamines that are metabolized into metanephrines (normetanephrine and metanephrine) and 3-methoxytyramine (3MT). Metanephrines are more sensitive biomarkers for the presence of paragangliomas than catecholamines and are also very useful for the establishment of malignancy [26]. According to the European Society of Endocrinology Clinical Practice Guidelines, the measurements of plasma or urinary metanephrines and 3MT are strongly recommended for the screening of metastasizing PPGLs [27]. Notably, this recommendation is based on the fact that after the resection of metanephrine- or 3MT-producing tumors, the increase in their levels should indicate progression (metastasis or recurrence) or new tumor development, but only on the condition that all tumor mass is completely removed. At the same time, only a few studies have investigated plasma or urine metanephrines and 3MT secretion in benign and metastasizing PPGLs. The results on the use of metanephrine concentration as a predictor of malignancy are controversial. Feng et al. reported both higher plasma and urine levels of metanephrine in metastasizing PHEOs [28]. Higher urinary metanephrine concentrations were also detected in patients with metastasizing PHEOs and PGLs than in patients with benign ones [29]. In contrast, a more recent study of Indian patients with PHEOs and PGLs revealed a lower secretion of plasma metanephrine in metastasizing cases [20]. The normetanephrine plasma level compared with the metanephrine one was found to be higher in metastasizing PPGLs [30]. Along with the increase in normetanephrine, elevated 3MT plasma levels were detected in metastasizing PPGLs, but a significant difference between metastasizing and benign tumors was shown only for 3MT [31]. High levels of 3MT are associated with *SDHB*-mutated PPGLs that are frequently characterized by the hypersecretion of norepinephrine and/or dopamine [32]. This explains the significant correlation of high 3MT levels with metastatic disease; however, these can also present in metastasizing PPGLs in the absence of *SDHB* mutations [31]. Nevertheless, measurements of 3MT concentrations after primary surgery are very helpful for the detection of tumor progression in most patients with germline *SDHB* mutations.

PPGLs can secrete not only catecholamines, but also various neuropeptides into the circulation. Neuropeptide Y (NPY) is widely expressed in the central and peripheral nervous system and is involved in the modulation of catecholamine secretion by adrenal chromaffin cells [33]. Increased plasma concentrations of neuropeptide Y (NPY) were detected in patients with PPGLs, particularly those with PHEOs [34]. A higher plasma level of NPY was observed in patients with metastasizing tumors than in those with benign tumors [35][36]. In a case of metastasizing extra-adrenal retroperitoneal paraganglioma, the NPY plasma level was decreased after primary tumor resection; however, its concentrations progressively increased during the postoperative period, coinciding with the documentation of metastases [37]. Conversely, decreased *NPY* gene expression was found in metastasizing PHEOs compared with benign ones [38][39]. Abnormal NPY plasma levels have not been reported for HNPGLs since these tumors are predominantly non-secreting. However, IHC analysis revealed high NPY expression in carotid body tumor tissues [40]. Further study of *NPY* gene expression in tissues of benign and metastasizing HNPGLs may help to develop its potential predictive values for these tumors.

Another candidate prognostic biochemical marker, neuron-specific enolase (NSE), was proposed by several studies [36][41]. Elevated serum levels of NSE can be found in metastasizing PHEOs along with plasma NPY levels. However, NSE

levels remain normal in benign tumors, which are also characterized by increased NSY concentrations. This finding indicates that the NSE serum level is more indicative for the prediction of malignancy risk.

Chromogranin A (CgA) is a well-known marker measured in neuroendocrine tumors that is commonly used for their diagnosis. PHEOs and secreting PGLs also express this protein and are characterized by diffuse positive IHC staining for CgA, while HNPGLs can have the focal expression pattern or be completely negative for CgA [42]. CgA is concentrated and stored in vesicles with other secretory peptides and is released by exocytosis from neuroendocrine cells. Elevated circulating levels of CgA have been associated with many neuroendocrine tumors as well as PPGLs [43]. The clinical sensitivity and specificity of the plasma CgA assay were close to those for metanephrines in laboratory diagnosis of PPGLs [44]. Circulating CgA was correlated with tumor mass; however, there were controversial results on its association with metastasizing PHEOs [45][46][47]. Generally, elevated plasma levels of CgA were detected in both benign and metastasizing PHEOs, but the highest concentrations were found in patients with metastases at the time of initial diagnosis. Moreover, high levels of CgA can be retained in patients with metastatic disease even after surgery on the primary tumor [46]. Hypersecretion of CgA was also observed in HNPGLs, including a metastasizing case; therefore, it can be used for the screening and follow-up of functional HNPGLs producing CgA (but not catecholamines) [48][49][50].

2.3. Genetic Markers

Among the main genetic features associated with a high risk of the development of metastasizing PPGLs is a germline mutation in the *SDHB* gene. Testing for the germline *SDHB* mutation in patients with PPGLs is recommended by Clinical Practice Guidelines [26][27]. According to a systematic review and a meta-analysis study, the pooled incidence risk of metastasizing PPGLs for the *SDHB* mutation carriers was 17% while the prevalence ranged from 13% to 23% [51]. Among the patients with HNPGLs, the reported incidence of metastasis reaches 83% in the groups of *SDHB* mutation carriers.

The germline mutation in the *SDHD* gene was also reported in metastasizing PPGLs; however, the risk of metastasis development in *SDHD* mutation carriers is significantly lower than in those with an *SDHB* mutation [52]. *SDHD* mutations are more frequently associated with HNPGLs and multiple tumors [53][54][55]. The pooled risk of incidence and prevalence of metastasizing PPGLs for *SDHD* mutation carriers was estimated as 8% and 3%, respectively [51]. The incidence risk of malignancy for patients with *SDHD*-mutated HNPGLs reaches 22.7%. The highest incidence risk of malignancy (100%, 4/4) was observed among patients with HNPGLs from the Dutch population; at the same time, no variants were found in the *SDHB* gene [56]. All these patients carried a founder mutation in the *SDHD* gene. Thus, this higher association of the *SDHD* mutation with malignancy compared with *SDHB*, which was found in most studies, can be explained by characteristics of the Dutch population.

Several studies reported germline mutations in other susceptibility genes for PPGLs, such as *FH* [57], *SLC25A11* [58], and *MDH2* [59], which were associated with aggressive tumor behavior. These genes are classified as cluster 1 TCA cycle-related associated with the pseudohypoxia subtype of PPGLs [60]. Moreover, tumors with mutations in these genes were clustered together with *SDHx*-mutated tumors demonstrating similar hypermethylation profiles [61][58][59][62]. This phenotype seems to be involved in tumor progression and mutations in the *FH*, *SLC25A11*, and *MDH2* genes along with *SDHB* and *SDHD* mutations can be considered to be a risk factor for PPGL malignancy. However, mutation frequency in these genes is rare and accounts for less than 1% [57][58][63]. Notably, alterations in *FH* and *SLC25A11* were found in HNPGLs but in non-metastasizing tumors [57][58][63].

ATRX is a frequent somatically mutated gene in PPGLs. The most frequent *ATRX* alterations have been observed in *SDHx*-mutated tumors, including metastasizing PPGLs [64]. Moreover, several studies showed that the somatic *ATRX* variant occurring with the *SDHB* mutation and/or *TERT* overexpression was an indicative marker of metastasizing tumors [65][66]. An important role of *ATRX* and telomere maintenance mechanisms during tumor progression was also confirmed by the presence of alternative lengthening of telomeres (ALT) in *ATRX*-mutated metastasizing PPGLs [67].

The Ki-67 protein is another important biomarker of tumor progression used in grading systems and prognosis prediction for several types of cancer [68]. It is also included in the pathological grading system GAPP for the estimation of metastatic potential in PPGLs. PPGLs usually have low proliferation activity with the Ki-67 score varying from 0% to 2%. However, elevated proliferation activity (over 2%) was observed in metastasizing PHEOs and PGLs [22][23][69]. Moreover, a series of studies have reported metastasizing PPGLs with the Ki-67 index of more than 4% [21][70]. Nevertheless, metastatic tumors can also have proliferation activity up to 2%, indicating the high specificity but low sensitivity of the method [71][72]. Recent research by Guo et al. showed an association between the Ki-67 index and the programmed death ligand 1 (PD-L1) expression in PPGLs [73]. Although PD-L1 expression was not significantly correlated with the presence of distant metastases, PD-L1 positivity in tumor cells with high Ki-67 may indicate that cells acquire the ability to escape the immune system, contributing to tumor growth, invasion, and metastasis [73][74]. The association of tumor progression with immune

evasion in PPGLs was confirmed by the fact that almost half of the metastasizing PPGLs expressed PD-L1 or PD-L2 [74]. Additionally, the TCGA project study found a positive correlation of the Ki-67 index with metastasizing PPGLs and its highest expression in *MAML3* fusion-positive tumors related to the Wnt signaling cluster. This indicates that the activation of the Wnt signaling pathway can promote tumor cell proliferation and progression of paragangliomas [75].

3. Conclusions

Prediction of malignancy for PPGLs is a great challenge. Many scientists have been searching for specific tumor features associated with malignancy risk in PPGLs for several decades. However, most of the published works have severe limitations, including small sample size, the rarity of metastasizing cases, tumor sets with a predominance of PHEOs, and lack of (or short) follow-up. Thus, although many parameters have been suggested as potential predictive factors of malignancy, the majority of them remain controversial. Nevertheless, a combination of different markers allows an increase in diagnostic accuracy for the identification of malignant potential in PPGLs. In our opinion, the main metastasis predictors for paragangliomas of all localizations are as follows: germline mutation in the *SDHB* gene, high Ki-67 index, and high plasma level of 3-methoxytyramine. **Figure 1** displays a probable algorithm for the diagnosis and follow-up of metastasizing PPGLs. The main potential markers for differentiating metastasizing and benign PPGLs are summarized in **Table 2**.

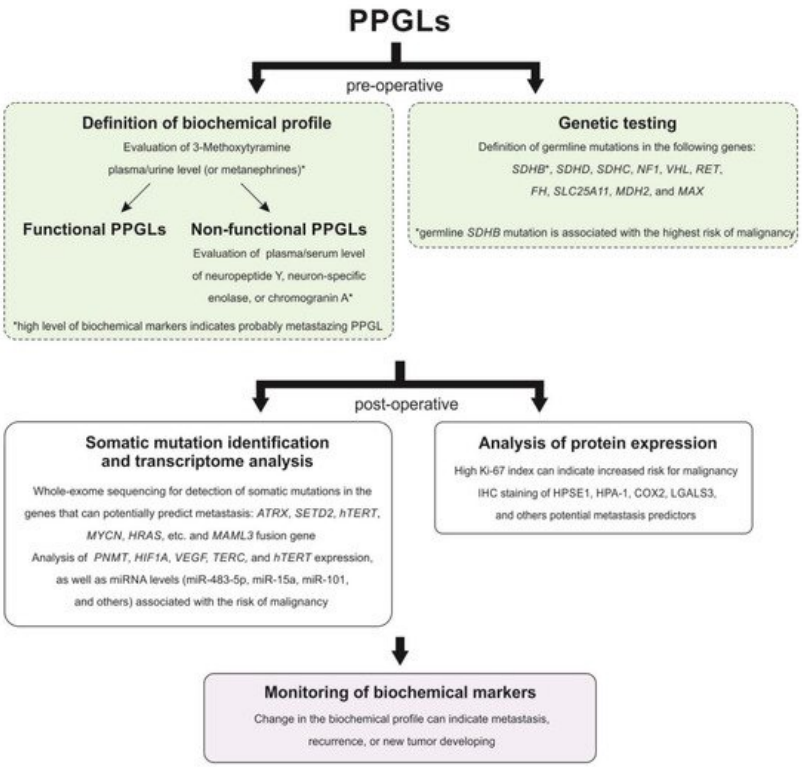

Figure 1. Possible scheme for the diagnosis and follow-up of metastasizing PPGLs.

Table 2. The main markers related to metastasizing PPGLs.

Potential Marker	Characteristics Associated with Malignancy
Histopathological markers	
Grading system for adrenal pheochromocytoma and paraganglioma (GAPP)	**Well-differentiated and moderately differentiated tumors**
Tumor size and weight	**On average, larger than 10 cm and more than 500 g**
Adrenal gland scaled score (PASS)	**≥4**
Ki-67 proliferation index	**>2%**
Sustentacular cells	**Cell density depletion or absent**
Galectin-3 (LGALS3)	**Increased expression detected using IHC staining**
Succinate dehydrogenase complex subunit B (SDHB)	**Negative or weak diffuse IHC staining**

Potential Marker	Characteristics Associated with Malignancy
Heparanase-1 (HPSE1)	Positive IHC staining
Cyclooxygenase-2 (COX2)	
Genetic markers	
Succinate dehydrogenase complex subunit B (SDHB)	Germline mutation
Succinate dehydrogenase complex subunit D (SDHD)	
Fumarate hydratase (FH)	
Solute carrier family 25 member 11 (SLC25A11)	
Malate dehydrogenase 2 (MDH2)	
ATRX chromatin remodeler (ATRX)	Somatic mutation
Histone-lysine N-methyltransferase SETD2 (SETD2)	
Telomerase reverse transcriptase (hTERT)	
Mastermind-like transcriptional coactivator 3 (MAML3)	Fusion gene
CpG island methylator phenotype (CIMP)	High CIMP
MicroRNA miR-15a	Downregulation
Phenylethanolamine N-methyltransferase (PNMT)	
MicroRNA miR-483-5p	Overexpression
MicroRNA miR-101	
MicroRNA miR-210	
MicroRNA miR-21-3p	
MicroRNA miR-183-5p	
Telomerase reverse transcriptase (hTERT)	
Biochemical markers	
Normetanephrine and 3-methoxytyramine	Increased plasma or urine level
Neuron-specific enolase (NSE)	Increased serum level

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