Challenges in Paragangliomas and Pheochromocytomas: from Histology to Molecular Immunohistochemistry

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Abstract
Abdominal paragangliomas and pheochromocytomas (PPGLs) are rare neuroendocrine tumors of the infradiaphragmatic paraganglia and adrenal medulla, respectively. Although few pathologists outside of endocrine tertiary centers will ever diagnose such a lesion, the tumors are well known through the medical community—possible due to a combination of the sheer rarity, their often-spectacular presentation due to excess catecholamine secretion as well as their unrivaled coupling to constitutional susceptibility gene mutations and hereditary syndromes. All PPGLs are thought to harbor malignant potential, and therefore pose several challenges to the practicing pathologist. Specifically, a responsible diagnostician should recognize both the capacity and limitations of histological, immunohistochemical, and molecular algorithms to pinpoint high risk for future metastatic disease. This focused review aims to provide the surgical pathologist with a condensed update regarding the current strategies available in order to deliver an accurate prognostication of these enigmatic lesions.

Keywords Pheochromocytoma · Abdominal paraganglioma · Review · Histology · PASS · GAPP · Immunohistochemistry · Molecular diagnostics

Introduction
Abdominal (sympathetic) paragangliomas and pheochromocytomas (PPGLs) are remarkable tumors, not only in terms of clinical presentation with a wide variety of symptoms derived from catecholamine production, but also with regards to the underlying tumor biology and its consequences for patient outcome. We now know that PPGLs constitute the most hereditable of all human tumors, with an established germline susceptibility event in approximately half of the patients [1–4]. Genetic events underlying the development of these lesions are noted in several different signaling pathways, of which mutations in genes regulating the tricarboxylic acid (TCA) cycle are particularly associated to metastatic disease [5]. Moreover, the former conception of “benign and “malignant” PPGL has now shifted towards a general appreciation that all tumors harbor malignant potential—thereby shifting the focus to a risk stratification approach to identify cases susceptible to future metastatic spread [1]. This focused review aims to cover the current histological, immunohistochemical and molecular approaches to pinpoint PPGLs at risk of dissemination, as well as to highlight the limitations. As parasympathetic paragangliomas of the head and neck region often are non-producing and clinically benign, they are not further discussed here [1].

Epidemiology and Clinical Workup
Traditionally considered a “one in a million” disease, PPGLs have shown a rising incidence during the last 40 years, from 1.4 per million person-years in 1977 to 6.6 in 2015, constituting a 4.8-fold increase [6]. The upsurge is largely attributable to smaller tumors in patients with few/no symptoms, suggesting that an intensified use of clinical imaging techniques might contribute to this increase. Indeed, most PPGLs are diagnosed incidentally following radiological investigations rather than via symptoms of catecholamine excess [7, 8]. PPGLs are usually visualized on conventional CT or MRI scans, although functional modalities such as 123I-meta-iodobenzylguanidine (123I-MIBG) scintigraphy or 68 Ga-DOTATOC positron emission tomography (PET) scans are often used due to their enhanced uptake in tumors.
often are needed to pinpoint the diagnosis [7, 9, 10]. In addition, plasma levels of chromogranin A and metanephrines are usually elevated [7, 11]. When localized to the primary site, cure rates are high if the tumor is surgically resected—however, treatment options for disseminated disease are limited [7, 12].

**Diagnostics**

As biopsies are generally not recommended for catecholamine-producing lesions, the diagnosis is typically made postoperatively after routine histopathological examination of the excised tumor [13]. Some baseline gross and microscopic characteristics are detailed in Fig. 1. Histologically, PPGLs often display a nested growth pattern (the so-called zellballen appearance) built-up by chief cells with abundant, basophilic cytoplasm. The tumor cells are usually surrounded by an arborizing network of thin blood vessels and supporting cells (“sustentacular cells”), which are recognizable only if immunohistochemical stains are applied (for example S100 and SOX10) (Fig. 1) [1]. The chief cells are strongly positive for neuroendocrine markers of both first (chromogranin A, synaptophysin) and second generation (ISL1, INSM1), and additional stains that may help distinguish PPGL include GATA3, tyrosine hydroxylase and dopamine beta-hydroxylase (Fig. 1) [1, 14–18]. Keratin expression is almost always absent, except for rare subtypes such as duodenal gangliocytic paragangliomas and cauda equina paragangliomas [14]. When assessing these lesions, the pathologist needs to consider many different aspects, including clinical information, primary tumor site, histology, the extent of invasion, the presence of vascular invasion, as well as the novel TNM staging system as dictated by the 8th edition of the American Joint Committee on Cancer (AJCC) Staging Manual [19, 20]. Interestingly, even though the TNM system for PPGL is newly adopted, the

![Fig. 1](image_url) **Fig. 1** Key gross, microscopic and immunohistochemical findings of PPGLs. A Macroscopic appearance of the resected adrenal with a 10 cm encapsulated pheochromocytoma exhibiting a fleshy cut surface with solid tan-colored areas. B Photomicrograph of hematoxylin–eosin (H&E)-stained tumor tissue at ×400 magnification revealing a nested growth pattern and an exceedingly well-vascularized stroma. C Chromogranin A immunostaining at ×400 magnification. Note the diffusely positive cytosolic staining. D Sustentacular cells visualized using an S100 staining. E The Ki-67 proliferation index can be used to assess the proliferative activity, and is also a key part of certain algorithms to assess the metastatic potential. F Positive SDHB immunohistochemistry argues against underlying SDHB, C, and D gene mutations, thereby indicating a lower risk of disseminated disease. G Core needle biopsy of a liver metastasis in a patient previously resected for a pheochromocytoma 2 years earlier, with metastatic tumor cells recognizable through their basophilic cytoplasm. These tumor cells were positive for neuroendocrine markers (not shown). Upper left portion depicts hepatocytes. H GATA3 immunohistochemistry displaying nuclear positivity. I Complete loss of sustentacular cells was noted, as evident by an S100 immunostaining. This phenomenon is often reported in metastatic cases.
staging seems to reflect the biological properties and clinical outcomes when applied on retrospective materials [21]. In addition, the pathologist could also consider to implement histological scoring algorithms in order to stratify the future risk of metastatic disease [19]. Although all PPGLs are considered to exhibit malignant potential, only 10–15% of pheochromocytomas and between 30 and 50% of abdominal paragangliomas will metastasize to non-chromaffin sites [7]. Therefore, there is a considerable distinction between exhibiting malignant potential and actually exhibiting clinical features of malignancy (i.e., to metastasize). A metastatic pheochromocytoma is illustrated in Fig. 1.

**Underlying Genetic Aberrancies: with Focus on Metastatic PPGL**

The genetics underlying the development of PPGLs is truly multifaceted. Following the identification of the von Hippel Lindau (VHL), Neurofibromatosis type (NF1) and Rearranged during transfection (RET) genes responsible for the VHL, NF1 and the multiple endocrine neoplasia type 2 (MEN2) multitumor syndromes respectively in which PPGL is a recurrent feature [22–25], the list has expanded considerably with the advent of comprehensive next-generation sequencing techniques. To date, germline alterations in more than 20 genes have been associated to the development of PPGL, and for this reason, the current WHO classification contains tumors with either germline or somatic mutations [29, 38, 41–44]. The pseudohypoxia cluster contains the highest proportion of metastatic PPGLs, and hence, this group of tumors deserves some increased attention [29, 41, 45]. Patients developing tumors that adhere to this cluster are usually young, which is due to an overall high frequency of constitutional mutations in susceptibility genes associated to PPGL development [5]. Interestingly, PPGLs within this cluster can be sub-stratified depending on whether or not gene mutations encoding enzymes responsible for propelling the tricarboxylic acid (TCA) cycle are present (Fig. 3, Fig. 4). TCA cycle aberrant PPGLs in general exhibit the highest risk of metastatic dissemination, which is due to the fact that the accumulation of onco-metabolites will inhibit the cellular effects of dioxygenases, a group of enzymes that catalyze the oxidation of various substrates via the conversion of α-ketoglutarate to succinate (Fig. 5) [46–50]. One important group of inhibited enzymes are the Ten-eleven translocation (TET) proteins responsible for genomic demethylation [49, 51, 52]. When TET enzymes are inhibited, the genome is more prone to hypermethylation of various regulatory regions, of which some have implication for the regulation of tumor development and metastatic spread (Fig. 5) [51–54]. On the other hand, pseudo-hypoxia-driven PPGLs without TCA cycle mutations are generally driven by mutations in the signaling networks that regulate hypoxia-inducible factor (HIF)-mediated transcription of target gene programs (Fig. 6). These events include inactivating mutations of the VHL, Egl-9 Family Hypoxia Inducible Factor 2 (EGLN2; encoding prolly hydroxylase 1, PHD1) and EGLN1 (encoding PHD2) genes, as well as activating EPAS1/HIF2-α mutations [40, 55–57]. The net result is halted degradation of HIF, leading to increased HIF signaling and promotion of angiogenesis and proliferation [57, 58]. However, while TCA cycle aberrant and TCA cycle non-aberrant PPGLs share dysregulation of HIF signaling, the latter group lacks the epigenetic dysregulation caused by TET enzyme inhibition. Given these molecular differences, TCA cycle–driven tumors are even more prone to metastatic spread than other PPGLs within the pseudo-hypoxia cluster, and there is probably a need to distinguish TCA cycle aberrant from TCA cycle non-aberrant PPGLs in terms of risk stratification [5, 59]. This is furthermore mirrored by syndromic manifestations, in which SDHx mutated PPGLs display a significant increased risk of disseminated disease, while VHL associated tumors rarely exhibit metastatic potential—although adhering to the same transcriptional cluster [5]. The Wnt cluster contains mostly pheochromocytomas with somatic fusions involving the Mastermind Like Transcriptional Coactivator 3 (MAML3) gene as well as Cold Shock Domain Containing E1 (CDSE1) mutations [29].
The MAML3 protein acts as a transcriptional coactivator of NOTCH pathway associated genes, and MAML3 fusions and MAML3 overexpression are recurrent features in various tumor types [60–62]. In PPGL, the fusion partners Upstream Binding Transcription Factor (UBTF) and Transcription Factor 4 (TCF4) promoter regions, stimulate constitutive overexpression of MAML3 [29]. The CDSE1 gene encodes an RNA binding protein involved in translational programming and RNA turnover [63, 64]. In PPGL, CDSE1 mutations are found on the somatic level and are expected to exhibit loss-of-function properties [29]. From a clinical standpoint, this Wnt expression cluster also contains PPGLs at risk of metastatic dissemination, probably arguing for tumor DNA screening of MAML3 gene fusions and CDSE1 mutations as an efficient way to identify additional high-risk cases that will be negative for other TCA cycle and pseudo-hypoxia-related aberrancies [5, 29].

Finally, the cortical admixture cluster is characterized by pheochromocytomas with NF1 somatic mutations as well as MEN2-related tumors with RET germline mutations. This cluster is enriched for adrenal cortical markers such as CYP11B1 and CYP21A2, which is probably due to the interspersion of adrenal cortical cells [29]. PPGL within this cluster exhibit a very low risk of metastatic spread.
Pinpointing Metastatic Potential using Histological Algorithms—Is it Possible?

Although not fully recommended by the current WHO classification, histological scoring systems are frequently used in the scientific literature when assessing the metastatic potential of PPGLs [1]. Even though the amount of verifying studies still is limited and the reproducibility debated, the intention of these algorithms is to provide the endocrine pathologist with schemes that might facilitate the identification of cases at risk of future dissemination. However, the limitations of these risk assessment models are mandatory to take into consideration when interpreting the outcome of each individual tumor.

The Pheochromocytoma of the Adrenal gland Scaled Score (PASS) was developed in 2002 as a method to identify pheochromocytomas with potential for aggressive behavior [65]. Dr. Thompson compared 50 “histologically malignant” and 50 “histologically benign” pheochromocytomas and identified key microscopic features that differed between groups. The algorithm is strictly histology-based and incorporates 12 different parameters that yield a score ranging from 0 to 20 points. One point each is given for the presence of nuclear hyperchromasia, profound nuclear pleomorphism, capsular invasion, or vascular invasion, whereas two points per parameter is given for large nests/compact growth, tumor necrosis, high cellularity, cellular monotony, tumor cell spindling, >3 mitoses per 10 high power fields, atypical mitoses, and extension into surrounding adipose tissue (Fig. 7). In the original cohort, a PASS score of 4 points or more indicated an increased risk of future aggressive behavior [65]. The scheme has been confirmed in several independent series [66–70], but not in other [71, 72]. Moreover, in terms of intra-observer variability, the PASS algorithm has been proven subpar—with different pathologists reaching different scores for a considerable proportion of cases [72, 73].

The PASS parameters listed above are classical attributes normally associated to malignant phenotypes in various human tumors. In this aspect, the PASS algorithm could be considered a “histological shotgun,” with a wide spread of microscopic criteria that will pinpoint cases at risk of spread—but also with an increased risk of false positives. Indeed, a recent meta-analysis of >800 pheochromocytomas with retrievable PASS scores and follow-up data [74] identified this algorithm as highly sensitive for the detection of metastatic cases with an ensuing high negative predictive value.
value. However, the specificity and corresponding positive predictive value were both low (Fig. 7). Therefore, the PASS algorithm could be viewed as a model to rule out metastatic potential (if the score is low), rather than to actually pinpoint cases that will behave malignant. Strikingly, the number of clinically benign pheochromocytomas with elevated PASS scores in the literature surpass the number of reported metastatic cases with similarly high PASS scores [74]. An example of the reduced specificity of the PASS algorithm is provided in Fig. 8, in which a resected pheochromocytoma with a recurrence-free follow-up time of 20 years displayed a pathological PASS score of 5, including vascular invasion, periadrenal invasion, capsular invasion, and nuclear pleomorphism. This serves as an illustration as to how PASS incorrectly may identify pheochromocytomas with little or no metastatic potential as potentially worrisome specimen in need of intensified follow-up. Moreover, specific genotype–phenotype observations of importance for MEN2A-associated PPGL have also been reported, as these tumors often present with large, irregular nests, focal tumor cell spindling, and an elevated Ki-67 index (Fig. 8) [75]. As of this, PPGL patients with germline RET mutations may exhibit alarming histological features, although these patients very rarely present with metastatic disease in the clinical setting. Therefore, it is imperative to take into consideration the medical history of each patient when conducting histological assessment of PPGLs—and the abovementioned example also serves to illustrate the importance of molecular genetics in complementing the pathology report in terms of accurate prognostication.
The Grading System for Adrenal Pheochromocytoma and Paraganglioma (GAPP) score was published in 2014 by Dr Kimura and co-workers, in which the authors studied 163 PPGLs, including 40 metastatic cases [76]. Unlike the PASS algorithm, the GAPP study incorporated both pheochromocytomas as well as abdominal paragangliomas, and aimed to highlight contributing factors indicating metastatic potential for this collective tumor group. Building on the PASS algorithm, the GAPP scoring system combines histological findings, immunohistochemistry and clinical information. More specifically, the GAPP score is retrieved by evaluating histological parameters (growth pattern, cellularity, presence of comedo-type necrosis, capsular and vascular invasion), the Ki-67 labelling index as well as the biochemical profile (catecholamine type) (Fig. 7). Noradrenergic PPGLs express low levels of the phenylethanolamine N-methyltransferase (PNMT) enzyme that converts norepinephrine to epinephrine, and reduced PNMT expression is in turn highly related to the pseudo-hypoxia signaling pathway [5, 77]. Therefore, norepinephrine secreting PPGLs are more likely to adhere to transcriptional clusters associated to metastatic behavior, which is also reflected in the GAPP score. The PPGLs are given a score ranging from 0 to 10 points, and subsequently graded as either well differentiated (WD, 0–2 points), moderately differentiated (MD, 3–6 points), or poorly differentiated (PD, 7–10 points). In this study, WD-PPGLs were all metastasis-free, while the metastatic proportion of cases was higher (and the disease-specific survival lower) in the MD and PD groups [76]. Moreover, time to a metastatic event decreased with increased GAPP scores. The GAPP scoring
The algorithm has only been reproduced in a few independent studies and is still a fairly young study in need of additional verification [72, 74, 78]. In the meta-analysis discussed above, the GAPP system exhibited a well-trusted “rule-out” function based on the excellent negative predictive value, similarly to what was shown for PASS (Fig. 7) [74]. However, the rather frequent finding of clinically benign PPGLs with scores ≥ 3 points makes the positive predictive value rather low, and hence puts a strain to the ability to truly identify cases at risk of metastatic spread.

**Early Immunohistochemical Analyses**

Given the association between increased mitotic activity and risk of metastatic spread, researchers early on turned to the Ki-67 proliferation marker in order to highlight cases at risk of dissemination. Studies seem to agree that clinically aggressive PPGLs are associated to higher Ki-67 indices, although overlaps exist [79–87]. Thus, there seem to be a large amount of scientific data that justified the inclusion of Ki-67 as one key parameter in the GAPP algorithm [76]. As Ki-67 is a part of the antibody lineup in most pathology laboratories and a stain that endocrine pathologists are acquainted to in terms of interpretation, this marker is thus a useful and potentially reproducible tool in the assessment of metastatic potential of PPGLs. Other early observations include the visualization of reduced amounts of sustentacular cells in metastatic PPGLs as visualized via S100 immunohistochemistry [87–92]. However, the S100 staining patterns might be heterogeneous and thereby hard to interpret, especially in larger tumors [89].

**Immunohistochemistry as Molecular Triaging**

The advent of modern next-generation analyses have revolutionized the ability to classify PPGLs, not only in terms of transcriptome clustering but also as a way to detect germline alterations in patients in need of genetic counselling and to pinpoint high-risk mutations in TCA cycle/pseudo-hypoxia-related PPGLs indicating higher risk of metastatic spread.
events. Even so, immunohistochemistry is still considered an efficient, cheap, and reproducible method to pinpoint cases in need of intensified molecular studies, as well as to evaluate the functional consequences of some genetic variants of uncertain significance detected through clinical genetics workup [93]. In terms of prognostication and clinical significance, SDHB immunohistochemistry is probably the most well-established marker to date. Following the detection of absent SDHB expression in hereditary PPGLs in patients with germline SDHB or SDHD mutations, several independent groups have verified the value of SDHB immunohistochemistry to pinpoint SDHx gene mutations occurring either on the somatic or germline level in PPGL [94–97]. The reason behind the ability of SDHB staining to pinpoint cases with either SDHB, C, or D subunit mutations stems from the fact that the succinate dehydrogenase enzyme complex is anchored to the mitochondrial inner membrane via the C and D subunits. Thus, mutational inactivation of SDHB, C, or D will cause a disruption of the entire complex, leading to absent SDHB immunoreactivity (Fig. 9) [98]. The scoring and interpretation of SDHB immunohistochemistry has been proved highly reproducible between pathologists and also a reliable tool in terms of detecting underlying SDHx gene mutations [99]. It should however be stressed that subsets of SHDB-immunodeficient PPGLs could display wild-type SDHx gene sequences, but instead exhibit SDHC promoter hypermethylation, alternatively VHL or NF1 gene mutations [99, 100]. In contrast, SDHA mutated PPGLs lose both SDHA and B immunoreactivity, and therefore, SDHA immunohistochemistry could complement the screening panel to detect rare PPGLs with SDHA mutations [99, 101]. SDHD immunostaining has also been assessed in PPGLs, in which positive immunoreactivity was observed in SDHx gene mutated cases—while wild-type cases stained negative. The reason for this paradoxal and inverted finding could be the potential de-masking of the SDHD epitope

Fig. 7 Schematic overview of the Pheochromocytoma of the Adrenal gland Scaled Score (PASS) and the Grading System for Adrenal Pheochromocytoma and Paraganglioma (GAPP) algorithms. Including parameters are listed, as well as the individual points given for each fulfilled criteria. Bottom row depicts the high negative predictive value of both algorithms as suggested by a recent meta-analysis, indicating that low PASS/GAPP scores are strongly associated to benign clinical courses, while elevated PASS/GAPP scores are recurrently reported in metastatic-free PPGLs—thereby limiting the value of these algorithms as “rule-in” tests. P point, E epinephrine, NP non-producing, NE norepinephrine

| Parameters | 1 p. each | 2 p. each |
|------------|-----------|-----------|
| Nuclear hyperchromasia | Profound nuclear pleomorphism | Capsular invasion |
| Vascular invasion | Large nests/compact growth | Tumor necrosis |
| High cellularity | Cellular monotony | Tumor cell spindling |
| >3 mitoses/10 HPFs | Atypical mitoses | Extension into adipose tissue |

| Parameters | 1 p. each | 0/0/1 p. |
|------------|-----------|-----------|
| Large and irregular cell nests | Pseudorosettes |
| Capsular or vascular invasion | Cellularity (low/medium/high) |
| Comedo-type necrosis | Ki-67 labelling index (<1%/1-3%/>3%) |
| Biochemical profile (E, NP, NE) | |

**PASS algorithm**

- **Designated tumor type**: Pheochromocytoma

- **Parameters**
  - Metastatic potential
  - PASS score ≥ 4 p.
  - Exceedingly rare

- **Clinical screening utility**
  - No metastatic potential

**GAPP algorithm**

- **Designated tumor type**: Pheochromocytoma and paraganglioma

- **Parameters**
  - Large and irregular cell nests
  - Pseudorosettes
  - Capsular or vascular invasion
  - Cellularity (low/medium/high)
  - Comedo-type necrosis
  - Ki-67 labelling index (<1%/1-3%/>3%)
  - Biochemical profile (E, NP, NE)

- **Clinical screening utility**
  - Metastatic potential
  - GAPP score ≥ 3 p.
  - Rare

- **No metastatic potential**
  - GAPP score < 3 p.
upon mutation-mediated disruption of the SDH complex [102]. Moreover, immunohistochemistry targeting fumarate hydratase (FH) has been proven as an efficient method to pinpoint rare FH gene germline mutations in PPGL [103], in turn coupled to the hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome [104].

Apart from the abovementioned markers used to pinpoint PPGLs associated to an aberrant TCA cycle, two markers

Fig. 8 Overstating the risk of aggressive behavior in PPGLs using current risk stratification algorithms. All images are routine hematoxylin–eosin stains. Images A–D depict a resected pheochromocytoma with a recurrence-free follow-up time of 20 years. A Pleomorphic features. B Capsular invasion. C Comedo-type necrosis. D Vascular invasion. The Pheochromocytoma of the Adrenal gland Scaled Score (PASS) was elevated (5 points); thus, the algorithm falsely identified metastatic potential in this instance. E, F A large proportion of PPGLs arising in MEN2A patients display large, irregular nests (E) and focal tumor cell spindling (F). Large nests is a parameter listed in both the PASS and the Grading System for Adrenal Pheochromocytoma and Paraganglioma (GAPP) algorithms, and tumor cell spindling is an additional PASS related parameter. From a clinical standpoint, pheochromocytomas in MEN2 patients very rarely metastasize

Fig. 9 Urinary bladder paraganglioma with an underlying SDHB mutation detected through immunohistochemistry. A Routine hematoxylin–eosin stain of a urinary bladder paraganglioma arising in a young female patient. B SDHB immunohistochemistry revealing absent tumor staining, while adjacent sustentacular cells and stromal components were positive. This staining pattern is highly indicative of an underlying SDHx gene mutation. C The SDHA immunostaining was positive, as indicated by a granular, cytoplasmic signal. D Regional lymph node exhibiting synchronous metastatic deposits. Clinical genetics counseling was initiated, and the patient was found to harbor a germline SDHB mutation
have shown promise to identify pseudo-hypoxia and kinase cluster associated tumors, respectively, namely carbonic anhydrase IX (CAIX) and MAX. CAIX is frequently found up-regulated in PPGL with underlying VHL gene mutations, and the identification of strong immunoreactivity in a PPGL might therefore be a way to identify VHL driven tumors with a lower (but not unneglectable) risk of aggressive behavior than TCA cycle aberrant PPGLs [105]. Similarly, rare cases of MAX mutated or gene rearranged PPGLs usually exhibit loss of MAX protein expression—adding yet another potential tool to the diagnostic workup of these lesions [27, 106]. In contrast, NF1 and RET immunohistochemistry cannot yet be recommended as a way to identify underlying NF1 and RET gene mutations, as both sensitivity and specificity have been found reduced in previous studies [107, 108].

Besides immunohistochemical analyses aiding in the context of underlying mutations, an additional marker of prognostic significance include chromogranin B (CHGB), which was the top downregulated gene in an expression study when stratifying for metastatic PPGLs [70]. The finding was reproduced using immunohistochemistry, with negative or low levels of CHGB immunoreactivity in metastatic PPGLs. Moreover, low preoperative plasma levels of CHGB were associated to higher PASS scores in the resected tumor. Thus, CHGB could possibly act as a preoperative marker of PPGLs with histological worrisome features.

Overall, a combined effort of histology and molecular immunohistochemistry is probably needed for the endocrine pathologist in order to better estimate the metastatic potential of each individual PPGL, including diagnostic and prognostic immunohistochemistry (Fig. 10). Moreover, the potential benefit of risk stratification algorithms has to be weighed against the risk of false positives and limited reproducibility, but could potentially be of value to identify cases with little risk of future dissemination.

Next-Generation Multi-OMICs Characterization—the Necessary Step

Recent next-generation sequencing studies of PPGLs have increased our understanding of molecular aberrancies that associate to metastatic potential. As previously discussed, gene fusions involving MAML3 and CDSE1 mutations are overrepresented in PPGL associated to the Wnt transcriptome cluster, and these tumors have a significantly higher metastatic rate than PPGL associated to the kinase cluster [5, 29]. These aberrancies are somatic and will thus not be identified during a clinical routine screening of germline DNA. Moreover, metastatic PPGLs also harbor somatic gene alterations of potential value for further investigations, such as mutations in transport and cell adhesion genes [109], recurrent Transcriptional regulator ATRX (ATRX) mutations [29, 110] as well as upregulated Telomerase reverse transcriptase (TERT) gene expression and TERT gene rearrangements [111, 112]. TERT gene aberrancies are common in various cancers and usually confer an increased TERT mRNA gene

![Fig. 10 Molecular immunohistochemistry of PPGLs. The endocrine pathologist needs to verify the diagnosis through a concerted action of histology and immunohistochemical markers that pinpoint the chromaffin cell origin. In terms of immunohistochemistry, a succinate dehydrogenase complex flavoprotein subunit B (SDHB) staining could pinpoint cases with underlying SHDx gene mutations and an increased risk of disseminated disease. Aberrant carbonic anhydrase IX (CAIX) and MYC associated factor X (MAX) immunostainings might indicate mutations in the von Hippel Lindau (VHL) or MAX genes, respectively, while the proliferation marker Ki-67 could be used to prognosticate the tumors further.](image-url)
output, which is thought to confer immortalization through the elongation of telomeric DNA for these tumor types [85, 112, 113]. ATRX encodes a chromatin remodeling protein, and mutations in this gene were intimately coupled to an alternative lengthening of telomeres, furthermore suggesting that the regulation of telomeric regions is crucial for metastatic PPGLs [29]. To add to the association between epigenetic regulators and metastatic potential, somatic mutations in SETD2 are a regulator of various cellular processes such as RNA splicing, DNA repair, DNA methylation and histone modification, and is considered a tumor suppressor in unrelated tumor types [114, 115]. In contrast, mutations in other components of the epigenetic machinery that governs chromatin remodeling are mostly found in metastatic-free PPGLs [31, 116]. Overall, screening for somatic gene aberrancies could complement routine histopathology and molecular immunohistochemical analyses in the hunt for PPGLs with metastatic potential, leading to more efficient pinpointing of high-risk cases.

In terms of epigenetic modifications, we know that especially TCA cycle aberrant PPGLs display unique methylation profiles on both global and gene-specific levels [52, 117, 118]. Although no clear-cut methylation panel for clinical usage in terms of prognostication exists, the association between hypermethylation, TCA cycle defects and metastatic PPGLs could have clinical implications in terms of treatment. For example, as O(6)-methylguanine-DNA methyltransferase (MGMT) constitutes an epigenetically silenced gene in SDHx mutated PPGLs, this could probably explain the partial effect of temozolomide in these cases [119].

Discussion

PPGLs are enigmatic lesions that pose a serious challenge even to tertiary center experts, not only in terms of clinical handling, but also (and perhaps; particularly) in terms of prognostication of each individual patient. The multifaceted genetic background and the complex association between histological hallmarks of malignancy and clinical evidence of metastatic spread requires a pathologist that can juggle with radiological, biochemical, genetic and histological parameters in order to prognosticate these lesions. Most importantly, an awareness of the limitations of each of these factors to pinpoint risk of disease dissemination is crucial, especially as overconfidence in any parameter might falsely indicate a placid PPGL as potentially aggressive. Thus, a modern endocrine pathologist needs to be updated not only on histological algorithms but also on the ever-dynamic genetic landscape and recent developments within the field of molecular testing. Moreover, additional coordinated multicenter research efforts will most likely be needed to fully dissect the molecular aberrancies that govern the malignant potential of PPGLs.

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Declarations

Conflicts of Interest Subsets of illustrations in this article have been featured during a lecture at the 2021 Endocrine Pathology Society Companion Meeting.

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