



Emerging molecular markers of metastatic pheochromocytomas and paragangliomas

Judith Goncalves, Charlotte Lussey-Lepoutre, Judith Favier, Anne-Paule Gimenez-Roqueplo, Luis Jaime Castro-Vega

► To cite this version:

Judith Goncalves, Charlotte Lussey-Lepoutre, Judith Favier, Anne-Paule Gimenez-Roqueplo, Luis Jaime Castro-Vega. Emerging molecular markers of metastatic pheochromocytomas and paragangliomas. *Annales d'Endocrinologie = Annals of Endocrinology*, 2019, 80, pp.159 - 162. <10.1016/j.ando.2019.04.003>. <hal-03484365>

HAL Id: hal-03484365

<https://hal.science/hal-03484365v1>

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

Dochead : Klotz communication 2019 : New insights into the pathophysiology and treatment of Neuroendocrine Tumors

Emerging Molecular Markers of Metastatic Pheochromocytomas and Paragangliomas

Marqueurs moléculaires émergents des phéochromocytomes et paragangliomes malins

Judith Goncalves^{1,2}, Charlotte Lussey-Lepoutre^{1,3}, Judith Favier^{1,2}, Anne-Paule Gimenez-Roqueplo^{1,2,4}, Luis Jaime Castro-Vega^{1,2*},

¹INSERM, UMR970, Paris-Cardiovascular Research Center, Equipe labellisée Ligue Contre le Cancer, F-75015, Paris, France

²Paris Descartes University, PRES Sorbonne Paris Cité, Faculté de Médecine, F-75006 Paris, France

³Sorbonne University Department of Nuclear Medicine, Pitié-Salpêtrière Hospital, F-75013, Paris, France

⁴Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Genetics Department, F-75015, Paris, France

Keywords: Pheochromocytoma, paraganglioma, metastasis, biomarkers

***Corresponding author**

Luis Jaime Castro-Vega, M.D., Ph.D.

INSERM, UMR970

Paris-Cardiovascular Research Center

F-75015, Paris, France

56 rue Leblanc, 75015 Paris, France

Phone: +33 1 53 98 80 45

e-mail: luis-jaime.castro-vega@inserm.fr

Summary

Metastatic pheochromocytoma/paraganglioma (PPGL) represents a major clinical challenge due to limitations in accurate diagnostic tools and effective treatments. Currently, patients classified at high-risk by means of clinical, biochemical and genetic criteria, require a lifelong monitoring, while it remains difficult to determine the metastatic potential of PPGL only on the basis of histopathological features. Thus, tumor molecular markers that improve the risk stratification of these patients are needed. In the past few years, we have witnessed an unprecedented molecular characterization of PPGL, which led to the emergence of promising candidate biomarkers predictive of metastatic behavior. Here, we briefly discuss these breakthroughs and provide some insights for the prospective implementation of molecular markers of metastatic PPGL in the clinical setting in years to come.

Résumé

Les phéochromocytomes et paragangliomes (PPGL) métastatiques posent de difficiles problèmes de diagnostic, pronostic, traitement et surveillance à long terme. La découverte de marqueurs moléculaires tumoraux est donc essentielle pour mieux définir le risque d'évolution métastatique des patients. Au cours des dernières années, nous avons assisté à une caractérisation moléculaire sans précédent des PPGL, qui a conduit à l'émergence de biomarqueurs candidats prometteurs, prédictifs du comportement métastatique. Dans cette revue, nous exposons et discutons ces avancées et l'application clinique potentielle de ces biomarqueurs moléculaires de PPGL métastatiques dans un futur proche.

Clinicopathological aspects of metastatic PPGL

Pheochromocytoma and paraganglioma (PPGL) are rare neuroendocrine tumors which arise in the sympathetic and parasympathetic paraganglia. Most of these tumors are not life-threatening and can be easily cured by surgery, whereas up to 20% may become metastatic¹. The clinical outcome of patients with metastatic PPGL is highly variable. More than 40% of patients present with metastases at the time of diagnosis while others up to 50 years afterwards^{2,3}. The overall 5-year survival rate of these patients varies between 34% and 60%⁴. Surgery can improve the prognosis⁵, but if the primary tumor is unresectable, rapid disease progression is observed³. Standard chemotherapeutic regimen with cyclophosphamide, vincristine, and dacarbazine, or radionuclide therapy with ¹³¹Iodine-radiolabelled metaiodobenzylguanidine only result in partial responses^{6,7}. Thus, clinical trials testing targeted therapies such as tyrosine kinase inhibitors are underway⁸.

Clinical, biochemical and genetic factors associated with metastatic PPGL comprise tumor location (extra-adrenal), large tumor size, high levels of plasma methoxytyramine and urinary dopamine, and germline mutations in *SDHB*, *FH* and *SLC25A11* genes^{1,9-12}. Carriers of mutations in other PPGL susceptibility genes have a lower risk^{13,14}. Higher mortality is associated with male sex, older age at the time of diagnosis, synchronous metastases, large tumor size, elevated dopamine, *SDHB* status and not undergoing primary tumor resection^{3,15}. Of note, the implementation of genetic testing in routine has greatly improved the management and clinical outcome of patients with *SDHx/VHL* mutations¹⁶. In marked contrast, there is no accurate mean to estimate the risk of developing a metastatic disease in patients with apparently sporadic PPGL, which would be helpful to guide their clinical management.

Histopathological features suggestive of metastatic behavior are the basis of the grade score systems PASS (Pheochromocytoma of the Adrenal Gland Scaled Score)¹⁷ and GAPP (The Grading system for Adrenal Pheochromocytoma and Paraganglioma)¹⁸. However low sensitivity and specificity¹⁹ as well as significant inter and intra-observer variabilities preclude

their implementation. The recent WHO classification of endocrine tumors pointed out that given the absence of reliable histopathologic criteria to distinguish “malignant” from “benign” PPGL, these two historically used terms should be abolished ²⁰. It was also emphasized that only the evidence of metastatic lesions, but not locally invasive or recurrent tumors defines metastatic PPGL²⁰. Since all PPGL presumably have a metastatic potential, molecular markers able to predict tumor aggressiveness are urgently needed.

Earliest molecular markers

Initial efforts made use of immunohistochemistry and RT-qPCR to evaluate the expression of candidate markers including cell proliferation antigen Ki-67/MIB-1 ^{21 22, 23}, c-Erb2 ^{23, 24}, angiogenic factors EPAS1 and VEGF ^{25 26}, CD34 (for assessment of vascular architecture) ²⁵, TERT ^{22, 27}, metastasis suppressors nm23-H1, TIMP-4, BRMS-1, TXNIP, CRSP-3 and E-Cad ^{28, 29}, epithelial-to-mesenchymal transition (EMT) inducers (Snail and Twist) ^{30, 31}, stemness factor OCT4 ³², activation of the mammalian target of rapamycin mTOR pathway ³³ and neuroendocrine gene transcripts in liquid biopsies ³⁴. In addition, fewer number of S-100+ sustentacular cells ³⁵, hypermethylation of the p16INK4A promoter ^{36, 37}, albeit modestly correlated with expression at mRNA and protein levels, and a CpG island methylator phenotype (CIMP) determined by examination of a panel of genes using pyrosequencing ³⁸ have been also suggested as markers of metastatic PPGL.

Although these exploratory studies indicated that metastatic PPGL may undergo increased proliferation and alterations of both differentiation and tissue architecture, they presented several methodological, technical and analytical limitations to draw conclusions. For instance, in most of them, the number of biosamples included was too small, and in the cases in which the genotype could be determined, the *SDHB* status appeared as a potential confounding factor. Markers evaluated by immunohistochemistry such as c-Erb2, CD34, OCT4 and S-100 could not be reproduced in subsequent independent series ³⁹⁻⁴². Dependency on the experience of the observer and antibodies differences may account for discrepancies when using this technique. Automated quantitative pathology imaging systems

should be used for a more objective evaluation of these markers. Variability of results based on RT-qPCR, on the other hand, is high, particularly when assessing low abundant transcripts such as the metastasis suppressor genes or the transcripts present in the circulation. Droplet digital PCR (ddPCR), a highly sensitive technique for assessment of gene expression, is recommended nowadays to circumvent this problem. Nonetheless, detection of transcripts such as *TERT* may render a false-positive result due to tumor lymphocytic infiltration. The potential utility of some of these earliest biomarkers as well as the discovery of new ones started to emerge upon the arrival of more comprehensive studies of large cohorts of PPGL intended to perform an unbiased molecular characterization.

Emergent molecular markers

Recent integrative genomic studies enabled to propose a molecular classification of PPGL into two main subtypes (cluster 1 and cluster 2) characterized by germline or somatic mutations in susceptibility genes or in recently identified drivers ⁴³. *SDHx/FH/SLC25A11*-related PPGL, which is the group of genetically defined tumors with the higher metastatic potential, exhibit global DNA hypermethylation, transcriptional signatures of EMT and angiogenesis/hypoxia signaling pathways and overexpression of miRNA cluster 182/96/183, miR-210 and miR-483. *VHL/EPAS1*-related tumors have intermediate methylation levels, a glycolytic profile, and similar to *SDHx*-related PPGL, also display a pseudohypoxic phenotype with overexpression of the hypoxic marker miR-210.

NF1/RET/TMEM127/MAX/HRAS/MET/FGFR1-related tumors are characterized by global hypomethylation, downregulation of miRNA cluster *DLK-MEG3* and overactivation of RAS/MAPK and IGF1 signaling pathways. A new molecular group was recently described, characterized by *MAML3* gene fusions and *CSDE1* somatic mutations linked to activation of the Wnt signaling pathway ⁴⁴.

Regarding metastatic PPGL, initial genomic studies reported that these tumors harbor high frequency of recurrent copy number alterations yet at large-scale ^{45, 46} as well as underexpression of >85% genes, probably linked to its undifferentiated pattern ⁴⁷. More

recently, the TCGA study suggested *MAML3* fusion gene, somatic mutations in *ATRX* and *SETD2* genes and high somatic mutation rate as novel markers of aggressive and/or metastatic PPGL⁴⁴. Expression data from this cohort further highlighted overactivation of cell cycle and regulation of actin cytoskeleton pathways⁴⁸ as well as overexpression of PDL-2 strongly correlated with genes reflecting an immune-exhausted T cell response⁴⁹. The TCGA study also confirmed that *SDHB* mutation, global hypermethylation and Ki-67 immunoreactivity were associated with poor clinical outcome. Given that no multivariate analyses were performed, it is difficult to ascertain the prognostic value of the newly identified markers compared to the *SDHB* status. In fact, *ATRX* mutations were initially reported to be frequent, yet not restricted to *SDHB*-related tumors⁵⁰. Interestingly, besides *ATRX* mutations, a hotspot mutation in the *TERT* promoter (C228T), which could not be captured by whole-exome sequencing analyses, was associated with the metastatic progression of *SDHB*-related tumors^{51, 52}.

Given that *TERT* and *ATRX* are mutually exclusive telomere maintenance mechanisms⁵³, it raised the question as to whether immortalization could be a prognostic factor, after all, most PPGL are indolent. To address this issue, we undertook a comprehensive analysis of immortalization in a series of 200 tumors collected by the French COMETE network⁵⁴. We showed that in fact only 20% of PPGL get immortalized by either telomerase activation or an alternative lengthening of telomeres (ALT) mechanism. Of note, different genomic alterations leading to the transcriptional activation of *TERT* comprising not only promoter mutation but also promoter hypermethylation and copy number gains, along with *ATRX* mutations, accounted for about 70% of metastatic PPGL and appeared as independent risk factors strongly associated with both metastasis-free and overall survivals. The receiver operating characteristic analysis yielded an area under the curve (AUC) of 0.84, demonstrating that these alterations perform more accurately than the *SDHB* status (AUC 0.70) on the ability to discriminate metastatic from non-metastatic PPGL. Although this study was devoid of independent validation, parallel observations support these findings^{50-52, 55, 56}. Notably,

immortalization-related mechanisms have also been reported to guide stratification and diagnosis in high risk neuroblastoma ⁵⁷ and glioma ⁵⁸, two other neural crest-derived tumors.

Given the potential of non-coding RNAs as prognostic markers ⁵⁹, we have also evaluated long non-coding RNAs (lncRNAs) using a mining-approach of transcriptome data from the same French COMETE cohort. Remarkably, we identified one putative lncRNA whose expression is dramatically reduced in metastatic tumors. This transcript accurately discriminated (AUC 0.95) metastatic from non-metastatic tumors with *SDHx* mutations and was significantly associated with short metastasis-free survival. Moreover, multivariate cox proportional hazards regression analyses for metastasis-free survival revealed this transcript as an independent risk factor associated with poor clinical outcome of *SDHx* mutation-carriers. Importantly, this transcript was further validated in an independent yet small series of *SDHx*-related tumors. It is expected that more classes of ncRNAs linked to metastatic PPGL will be discovered using RNA-seq approaches in the years to come.

Additionally, two powerful international collaborative studies carried out at centers of the European Network for the Study of Adrenal Tumors (ENS@T) led to the discovery of additional informative markers. One of these studies consisted on the analysis of compiled miRNA profiling data from TCGA, COMETE and Spanish cohorts (450 tumors; follow-up period 35 years) and enabled the identification of a six-miRNA metastatic signature strongly correlated with time to progression, irrespective of *SDHB* status. In fact, combined analysis of two of those miRNAs yielded an AUC of 0.80 which was largely better than the *SDHB* status (AUC 0.63) in this study. Moreover, this signature was validated in an independent series of 49 formalin-fixed paraffin-embedded (FFPE) tissue specimens and partially retrieved as circulating miRNA in patients' liquid biopsies, particularly during the progressive phase of the metastatic disease (unpublished personal observation). The second study consisted on the analysis of compiled methylome data from 277 tumors ⁶⁰ including 48 metastatic PPGL. The authors successfully identified hypermethylation in the promoter of the *RDBP* (negative elongation factor complex member E) gene as a promising biomarker of

metastatic PPGL significantly associated with time to progression, even after correcting for *SDHB* genotype. Further validation was provided in an independent series of 33 FFPE tumors including 19 metastatic. Although this finding was not reproduced by another group, it should be noted that this second study analyzed a smaller PPGL cohort (n=39), including 10 metastatic tumors only from cluster 1 ⁶¹.

Conclusions and future perspectives

Current international guidelines for the clinical management of patients operated for a PPGL recommend assaying plasma or urinary metanephrines every year to screen for local or metastatic recurrences or new tumors and define a category of patients at high-risk of developing a metastatic disease (young patients and those with a genetic disease, a large tumor and/or a paraganglioma) for whom a lifelong annual follow-up is required ⁶². It is important to note that while genetic testing readily improve the outcome of patients *SDHB* mutation-carriers which are at high risk ¹⁶, an important proportion of patients with sporadic tumors can also develop a metastatic disease.

Earliest studies of candidate prognostic markers had several selection biases including small cohorts, undetermined genetic predispositions (some studies were conducted before the advent of genetic testing), variable or unreported follow-up times and regimen of surveillance, different definitions of metastatic PPGL and no statistical analyses to adequately assess the outcomes of patients. In the last few years, molecular characterization of large series of PPGL with well-annotated clinical and genetic data, led to the discovery of promising markers, able to predict the metastatic behavior of both *SDHB*-mutated and sporadic tumors and so there is exciting opportunity to further leverage these advances. The time is ripe for launching prospective multicentre studies in a real-life clinical scenario, aimed at comparing the diagnostic/prognostic performance of those novel markers in order to choose the best discriminators. Thus, a final prognostication model of metastatic PPGL could be developed by combining the most reliable clinical, genetic and molecular risk factors.

It is expected that tumor biomarkers may become integral to management strategies in metastatic patients in the era of cancer precision medicine. However, as reliable biomarkers arrive, new challenges are expected. For instance, assessment of molecular markers in cases with multiple tumors would be difficult in routine practice. Moreover, likewise other human cancers, metastatic PPGL display a high degree of genetic⁶³ and epigenetic⁶¹ heterogeneity. Therefore, a single biopsy may not capture the complete molecular landscape of an evolving tumor. To evaluate disease progression, assessment of molecular markers in liquid biopsies will be more appropriate. In this regard, circulating miRNAs and detection of individual mutations in cell-free DNA by means of ddPCR already holds significant promise. We believe that implementation of molecular markers of metastatic PPGL in the near future will be useful to improve risk stratification, to guide patient care, and consequently to reduce the associated medico-economic costs of long-term surveillance.

Acknowledgements

Work in the Genetics and Metabolism of Rare Cancers Lab is supported by past and current grants from European Commission FP7 Research and Innovation Funding Program for 2007-2013 (n° 259735), Horizon 2020 (n° 633983); Institut National du Cancer and Direction Générale de l'Offre de Soins (DGOS), Programme de Recherche Translationnelle en cancérologie (PRT-K 2014, COMETE-TACTIC, INCa_DGOS_8663); Agence Nationale de la Recherche (ANR-2011-JCJC-00701 MODEOMAPP); Alliance nationale pour les sciences de la vie et de la santé (AVIESAN); Plan Cancer: Appel à projets Epigénétique et Cancer 2013 (EPIG201303 METABEPIC), the Cancer Research for Personalized Medicine - CARPEM project (Site de Recherche Intégré sur le Cancer - SIRIC) and The Paradifference Foundation. The group is supported by the Ligue Nationale contre le Cancer (Equipe Labellisée). We apologize to all colleagues whose important contributions could not be cited due to space limitations.

References

1. Ayala-Ramirez, M. et al. Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. *J Clin Endocrinol Metab* **96**, 717-25 (2011).
2. Hamidi, O. et al. Malignant Pheochromocytoma and Paraganglioma: 272 Patients Over 55 Years. *J Clin Endocrinol Metab* **102**, 3296-3305 (2017).
3. Hamidi, O. et al. Outcomes of patients with metastatic phaeochromocytoma and paraganglioma: A systematic review and meta-analysis. *Clin Endocrinol (Oxf)* **87**, 440-450 (2017).
4. Pacak, K. et al. Pheochromocytoma: recommendations for clinical practice from the First International Symposium. October 2005. *Nat Clin Pract Endocrinol Metab* **3**, 92-102 (2007).
5. Roman-Gonzalez, A. et al. Impact of Surgical Resection of the Primary Tumor on Overall Survival in Patients With Metastatic Pheochromocytoma or Sympathetic Paraganglioma. *Ann Surg* **268**, 172-178 (2018).
6. Niemeijer, N.D., Alblas, G., van Hulsteijn, L.T., Dekkers, O.M. & Corssmit, E.P. Chemotherapy with cyclophosphamide, vincristine and dacarbazine for malignant paraganglioma and pheochromocytoma: systematic review and meta-analysis. *Clin Endocrinol (Oxf)* **81**, 642-51 (2014).
7. Baudin, E. et al. Therapy of endocrine disease: treatment of malignant pheochromocytoma and paraganglioma. *Eur J Endocrinol* **171**, R111-22 (2014).
8. Nolting, S., Grossman, A. & Pacak, K. Metastatic Phaeochromocytoma: Spinning Towards More Promising Treatment Options. *Exp Clin Endocrinol Diabetes* **127**, 117-128 (2019).
9. Eisenhofer, G. et al. Plasma methoxytyramine: a novel biomarker of metastatic pheochromocytoma and paraganglioma in relation to established risk factors of tumour size, location and SDHB mutation status. *Eur J Cancer* **48**, 1739-49 (2012).
10. Amar, L. et al. Succinate dehydrogenase B gene mutations predict survival in patients with malignant pheochromocytomas or paragangliomas. *J Clin Endocrinol Metab* **92**, 3822-8 (2007).
11. Castro-Vega, L.J. et al. Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum Mol Genet* **23**, 2440-6 (2014).
12. Buffet, A. et al. Germline Mutations in the Mitochondrial 2-Oxoglutarate/Malate Carrier SLC25A11 Gene Confer a Predisposition to Metastatic Paragangliomas. *Cancer Res* **78**, 1914-1922 (2018).
13. Bausch, B., Borozdin, W., Neumann, H.P. & European-American Pheochromocytoma Study, G. Clinical and genetic characteristics of patients with neurofibromatosis type 1 and pheochromocytoma. *N Engl J Med* **354**, 2729-31 (2006).
14. Crona, J. et al. Genotype-phenotype correlations in pheochromocytoma and paraganglioma. *Endocr Relat Cancer* (2019).
15. Turkova, H. et al. Characteristics and Outcomes of Metastatic Sdhb and Sporadic Pheochromocytoma/Paraganglioma: An National Institutes of Health Study. *Endocr Pract* **22**, 302-14 (2016).
16. Buffet, A. et al. Positive Impact of Genetic Test on the Management and Outcome of Patients With Paraganglioma and/or Pheochromocytoma. *J Clin Endocrinol Metab* **104**, 1109-1118 (2019).
17. Thompson, L.D. Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases. *Am J Surg Pathol* **26**, 551-66 (2002).
18. Kimura, N. et al. Pathological grading for predicting metastasis in phaeochromocytoma and paraganglioma. *Endocr Relat Cancer* **21**, 405-14 (2014).

19. Stenman, A., Zedenius, J. & Juhlin, C.C. The Value of Histological Algorithms to Predict the Malignancy Potential of Pheochromocytomas and Abdominal Paragangliomas-A Meta-Analysis and Systematic Review of the Literature. *Cancers (Basel)* **11** (2019).
20. Lloyd RV OR, K.G., Rosai J. WHO classification of tumours: pathology and genetics of tumours of endocrine organs. (2017).
21. Clarke, M.R., Weyant, R.J., Watson, C.G. & Carty, S.E. Prognostic markers in pheochromocytoma. *Hum Pathol* **29**, 522-6 (1998).
22. Elder, E.E. et al. KI-67 AND hTERT expression can aid in the distinction between malignant and benign pheochromocytoma and paraganglioma. *Mod Pathol* **16**, 246-55 (2003).
23. Tavangar, S.M. et al. Immunohistochemical expression of Ki67, c-erbB-2, and c-kit antigens in benign and malignant pheochromocytoma. *Pathol Res Pract* **206**, 305-9 (2010).
24. Castilla-Guerra, L. et al. Expression and prognostic value of c-erbB-2 oncogene product in human phaeochromocytomas. *Histopathology* **31**, 144-9 (1997).
25. Favier, J., Plouin, P.F., Corvol, P. & Gasc, J.M. Angiogenesis and vascular architecture in pheochromocytomas: distinctive traits in malignant tumors. *Am J Pathol* **161**, 1235-46 (2002).
26. Salmenkivi, K., Heikkilä, P., Liu, J., Haglund, C. & Arola, J. VEGF in 105 pheochromocytomas: enhanced expression correlates with malignant outcome. *APMIS* **111**, 458-64 (2003).
27. Boltze, C. et al. Expression profile of the telomeric complex discriminates between benign and malignant pheochromocytoma. *J Clin Endocrinol Metab* **88**, 4280-6 (2003).
28. Ohta, S. et al. Downregulation of metastasis suppressor genes in malignant pheochromocytoma. *Int J Cancer* **114**, 139-43 (2005).
29. Saffar, H. et al. Expression of galectin-3, nm-23, and cyclooxygenase-2 could potentially discriminate between benign and malignant pheochromocytoma. *Am J Clin Pathol* **135**, 454-60 (2011).
30. Waldmann, J. et al. Expression of the transcription factor snail and its target gene twist are associated with malignancy in pheochromocytomas. *Ann Surg Oncol* **16**, 1997-2005 (2009).
31. Hayry, V. et al. High frequency of SNAIL-expressing cells confirms and predicts metastatic potential of phaeochromocytoma. *Endocr Relat Cancer* **16**, 1211-8 (2009).
32. Alexander, R.E., Cheng, L., Grignon, D.J. & Idrees, M. Cytoplasmic staining of OCT4 is a highly sensitive marker of adrenal medullary-derived tissue. *Am J Surg Pathol* **37**, 727-33 (2013).
33. Chaux, A. et al. Immunohistochemical evidence of dysregulation of the mammalian target of rapamycin pathway in primary and metastatic pheochromocytomas. *Urology* **80**, 736 e7-12 (2012).
34. Peczkowska, M. et al. The clinical utility of circulating neuroendocrine gene transcript analysis in well-differentiated paragangliomas and pheochromocytomas. *Eur J Endocrinol* **176**, 143-157 (2017).
35. Unger, P. et al. S100 protein-positive sustentacular cells in malignant and locally aggressive adrenal pheochromocytomas. *Arch Pathol Lab Med* **115**, 484-7 (1991).
36. Kiss, N.B. et al. Methylation of the p16INK4A promoter is associated with malignant behavior in abdominal extra-adrenal paragangliomas but not pheochromocytomas. *Endocr Relat Cancer* **15**, 609-21 (2008).
37. Kiss, N.B. et al. Acquired hypermethylation of the P16INK4A promoter in abdominal paraganglioma: relation to adverse tumor phenotype and predisposing mutation. *Endocr Relat Cancer* **20**, 65-78 (2013).
38. Geli, J. et al. Global and regional CpG methylation in pheochromocytomas and abdominal paragangliomas: association to malignant behavior. *Clin Cancer Res* **14**, 2551-9 (2008).
39. de Krijger, R.R. et al. Prognostic value of p53, bcl-2, and c-erbB-2 protein expression in phaeochromocytomas. *J Pathol* **188**, 51-5 (1999).
40. Oudijk, L. et al. Vascular pattern analysis for the prediction of clinical behaviour in pheochromocytomas and paragangliomas. *PLoS One* **10**, e0121361 (2015).

41. Oudijk, L. et al. Immunohistochemical expression of stem cell markers in pheochromocytomas/paragangliomas is associated with SDHx mutations. *Eur J Endocrinol* **173**, 43-52 (2015).
42. Białas M, O.K., Dyduch G, Ciesielska-Milian K, Buziak M, Hubalewska-Dydejczyk A, Sobrinho-Simoes M. Neuroendocrine markers and sustentacular cell count in benign and malignant pheochromocytomas - a comparative study. *Pol J Pathol* **64**, 129-135 (2013).
43. Castro-Vega, L.J., Lepoutre-Lussey, C., Gimenez-Roqueplo, A.P. & Favier, J. Rethinking pheochromocytomas and paragangliomas from a genomic perspective. *Oncogene* **35**, 1080-9 (2016).
44. Fishbein, L. et al. Comprehensive Molecular Characterization of Pheochromocytoma and Paraganglioma. *Cancer Cell* **31**, 181-193 (2017).
45. Sandgren, J. et al. Recurrent genomic alterations in benign and malignant pheochromocytomas and paragangliomas revealed by whole-genome array comparative genomic hybridization analysis. *Endocr Relat Cancer* **17**, 561-79 (2010).
46. Petri, B.J. et al. Frequent loss of 17p, but no p53 mutations or protein overexpression in benign and malignant pheochromocytomas. *Mod Pathol* **21**, 407-13 (2008).
47. Brouwers, F.M. et al. Gene expression profiling of benign and malignant pheochromocytoma. *Ann N Y Acad Sci* **1073**, 541-56 (2006).
48. Suh, Y.J., Choe, J.Y. & Park, H.J. Malignancy in Pheochromocytoma or Paraganglioma: Integrative Analysis of 176 Cases in TCGA. *Endocr Pathol* **28**, 159-164 (2017).
49. Pinato, D.J. et al. Programmed cell death ligands expression in phaeochromocytomas and paragangliomas: Relationship with the hypoxic response, immune evasion and malignant behavior. *Oncoimmunology* **6**, e1358332 (2017).
50. Fishbein, L. et al. Whole-exome sequencing identifies somatic ATRX mutations in pheochromocytomas and paragangliomas. *Nat Commun* **6**, 6140 (2015).
51. Liu, T. et al. The activating TERT promoter mutation C228T is recurrent in subsets of adrenal tumors. *Endocr Relat Cancer* **21**, 427-34 (2014).
52. Papathomas, T.G. et al. Telomerase reverse transcriptase promoter mutations in tumors originating from the adrenal gland and extra-adrenal paraganglia. *Endocr Relat Cancer* **21**, 653-61 (2014).
53. Reddel, R.R. Telomere maintenance mechanisms in cancer: clinical implications. *Curr Pharm Des* **20**, 6361-74 (2014).
54. Job, S. et al. Telomerase Activation and ATRX Mutations Are Independent Risk Factors for Metastatic Pheochromocytoma and Paraganglioma. *Clin Cancer Res* **25**, 760-770 (2019).
55. Svahn, F. et al. Telomerase reverse transcriptase promoter hypermethylation is associated with metastatic disease in abdominal paraganglioma. *Clin Endocrinol (Oxf)* **88**, 343-345 (2018).
56. Dwight, T. et al. TERT structural rearrangements in metastatic pheochromocytomas. *Endocr Relat Cancer* **25**, 1-9 (2018).
57. Ackermann, S. et al. A mechanistic classification of clinical phenotypes in neuroblastoma. *Science* **362**, 1165-1170 (2018).
58. Pekmezci, M. et al. Adult infiltrating gliomas with WHO 2016 integrated diagnosis: additional prognostic roles of ATRX and TERT. *Acta Neuropathol* **133**, 1001-1016 (2017).
59. Ching, T. et al. Pan-Cancer Analyses Reveal Long Intergenic Non-Coding RNAs Relevant to Tumor Diagnosis, Subtyping and Prognosis. *EBioMedicine* **7**, 62-72 (2016).
60. de Cubas, A.A. et al. DNA Methylation Profiling in Pheochromocytoma and Paraganglioma Reveals Diagnostic and Prognostic Markers. *Clin Cancer Res* **21**, 3020-30 (2015).
61. Backman, S. et al. Global DNA Methylation Analysis Identifies Two Discrete clusters of Pheochromocytoma with Distinct Genomic and Genetic Alterations. *Sci Rep* **7**, 44943 (2017).
62. Plouin, P.F. et al. European Society of Endocrinology Clinical Practice Guideline for long-term follow-up of patients operated on for a phaeochromocytoma or a paraganglioma. *Eur J Endocrinol* **174**, G1-G10 (2016).

63. Crona, J. et al. Spatiotemporal Heterogeneity Characterizes the Genetic Landscape of Pheochromocytoma and Defines Early Events in Tumorigenesis. *Clin Cancer Res* **21**, 4451-60 (2015).