PHEOCHROMOCYTOMA-PARAGANGLIOMA: TRANSLATIONAL APPROACH FROM GENETICS TO CLINICAL NEUROENDOCRINOLOGY

S. Lider^{1,2,*}, M. Gheorghiu^{1,2}

¹“C.I. Parhon” National Institute of Endocrinology, ²“C. Davila” University of Medicine and Pharmacy, Dept. of Endocrinology, Bucharest, Romania

Abstract

In the latest decade, translational medicine has played an important role. In neuroendocrine tumor field, genetic results are associated with clinical and paraclinical disease implications. This translational knowledge allows a faster action in some mutation types with aggressive outcome. In the following pages we will make a review about actualities in translational approach from genetics to clinical neuroendocrinology.

INTRODUCTION

Pheochromocytomas and paragangliomas (PPGLs) are rare pathologies and they are mostly benign. According to the World Health Organization classification (2017) of endocrine tumors, pheochromocytomas (PCCs) are tumors of chromaffin cells that arise from the adrenal medulla, whereas paragangliomas (PGLs) are neural crest-derived neuroendocrine tumors that can be found at any level of extra-adrenal paraganglia (from the skull base to the pelvis) (1).

Genetic testing for pheochromocytoma and paraganglioma allows an early detection of hereditary syndromes and facilitates a close follow-up of high-risk patients.

Approximately 30–40% of PPGLs are now known to be hereditary. Germline mutations in genes that encode the SDH complex (SDHA, SDHB, SDHC, SDHD, SDHAF2) represent half of all hereditary diseases, followed by germline mutations associated with von Hippel-Lindau syndrome (VHL), multiple endocrine neoplasia type 2 (RET), neurofibromatosis type 1 (NF1), and some rare mutations in a number of genes (EPAS1, TMEM127, MAX, KIF1B β , PHD2, FH, MDH2, MEN1) (2).

In a retrospective study from 2017, assessed on 129 patients diagnosed with PPGL between 2000-

2015, it has been demonstrated that the patient referral to a geneticist for genetic counseling has significantly increased. However, only two-thirds of patients were followed up with genetic counselors (3).

In the latest years, in the field of neuroendocrine tumors, translational medicine has played an important role. Genetic results are associated with clinical and paraclinical disease implications. This translational knowledge allows a holistic view of the disease and a faster action in some mutation types with aggressive outcome.

1.1 Updates in translational neuroendocrinology - from genetics to clinical and biochemical features

Regarding clinical and paraclinical features correlated to mutation type, Rami Alrezk *et al.* (4) describe, in a review published in 2018, four PPGLs phenotypes:

A. Silent phenotype, with clinically asymptomatic patients. Frequently this phenotype is associated with PGL located to head and neck (HNPPGL) and with SDHx syndromes.

B. Biochemically pseudosilent phenotype- This usually happens in patients with very small (less than 5-7mm) PPGLs. Catecholamine levels are normal, or borderline to the upper limit of normal.

C. Noradrenergic phenotype - Usually the tumors is located outside adrenal gland. Patients present less frequently paroxysmal symptomatology; most commonly they present tachycardia and hypertension. This phenotype is associated with increased levels of noradrenaline and is commonly reported in the cluster 1/ pseudohypoxia group of genes, including VHL and SDHx mutations.

D. Adrenergic phenotype-associated with paroxysmal symptomatology and high levels of metanephrines. This phenotype is commonly seen in

*Correspondence to: Sofia Lider, “C.I. Parhon” National Institute of Endocrinology, 34-36 Aviatorilor Blvd., 011863, Bucharest, Romania, E-mail: sofia.lider@yahoo.com

the cluster 2/kinase signaling group.

E. Dopaminergic phenotype – PPGLs located in the neck and head region. This phenotype is characterized by high levels of DA/3-methoxytyramine with normal or near-normal levels of metanephrines and normetanephrines. They are usually associated with SDHx mutations especially in SDHB(4).

1.2 Updates in translational neuro-endocrinology: from genetics to imagistic

After biochemical assessment, imagistic evaluation is an indispensable approach.

The study published in 2015 by Janssen I *et al.* demonstrates that ^{68}Ga -DOTATATE PET/CT has proven to be elective - with a 98.6% overall detection rate in patients with SDHB metastatic PPGL, superior to anatomic imaging (CT/MRI) or other functional scans (18F-FDG, 18FFDOPA, and 18F-FDA PET/CT (5).

In specific mutations, ^{68}Ga -DOTATATE PET/CT resulted inferior in the evaluation of patients with polycythemia/PPGL including both HIF2A and PHD1-related tumors, FH or MAX mutations. In these patients with polycythemia associated with PPGL, the combination of 18F-FDOPA PET/CT and 18F-FDA PET/CT has better results with a lesion-base detection rate of ~98%, vs. 35.3% for the ^{68}Ga -DOTATATE PET/CT (4, 5).

A review from 2018 shows that in pediatric population, ^{68}Ga -DOTATATE PET/CT has shown to be superior in children with SDHx-related PPGL with a detection rate of 98.4% when compared to 18F-FDG PET/CT (4).

As a new perspective in neuroendocrine tumors therapy, ^{177}Lu -DOTATATE (LutatheraR) has been recently approved by the food and drug administration for the treatment of advanced midgut NETs. Applications of Lu-DOTATE in pheochromocytomas and paragangliomas have been demonstrated in patients with mediastinal or HNPGL and with inoperable HNPGL with promising results on tumor dimensions and symptoms (6).

1.3 Updates in translational neuroendocrinology: from genetics to immunohistochemistry

Immunohistochemistry is the last step in diagnosing and establishing disease outcome. A new association between tumor immunohistochemical particularities and SDH cluster mutation was described in a study from 2018 (1).

Negative staining of SDHB PPGLs occurs in the case of a bi-allelic inactivation of any of the components of SDH (that is, SDHA, SDHB, SDHC, SDHD or SDHAF2) while positive staining occurs in sporadic disease.

In tumors caused by SDHD germline mutation, the cytoplasm may show a pale and diffuse blush and is different from the granular mitochondrial pattern and should be interpreted as a negative pattern of staining (1).

HIF-1 α stabilization was associated with SDHx-mutations in HNPGLs, not in PCCs/PGLs. However, 25% of SDHx-HNPGLs lacked HIF-1 α positive cells (7). In some cases, pheochromocytomas associated with VHL syndrome express proteins related to the hypoxic response, like carbonic anhydrase IX (8).

CNTN4 protein expression is associated with malignant behaviour in PPGLs (9).

New markers supposed to be expressed in PPGLs including: cyclooxygenase 2, heat-shock protein 90, telomerase activity through hTERT, vascular endothelial growth factor, cell surface glycoprotein CD44, human epidermal growth factor-2, and insulin-like growth factor 1 receptor are in experimental stage (1).

1.4. Epigenetics and new approaches in clinical genetics

Epigenetics is another aspect that leads to multiple questions about the pattern of new diseases. Changes in DNA molecule can be caused by: maternal nutrition during pregnancy general famine, early prenatal stress, and smoking. There are some evidence that epigenetic alterations may drive the development of pheochromocytoma and paraganglioma (10).

A recent case report described epigenetic inactivation of SDHC by promoter hypermethylation in a patient with multiple paragangliomas. The protein-level expression of both SDHB and SDHC was abolished. The same mechanism of disease has been described in patients with Gastrointestinal Stromal Tumors and the Carney triad (11).

Job Set *et al.* (12) demonstrated a new epigenetic alteration-ATRX mutation. Located on the X chromosome, ATRX encodes the transcriptional regulator ATRX. ATRX plays a role in the histone deposition in telomeres, chromosome segregation in the cell cycle and transcription regulation (4). Inactivation of ATRX leads to activation of an alternative lengthening of telomeres (ALT) pathway. Assessment

of ATRX mutations and telomerase activation could be used to identify metastatic PPGLs, particularly in aggressive tumors (4).

Another histone modifier, KMT2D, has been found recurrently mutated in pheochromocytomas (4).

Toledo *et al.* analyzed patients' samples with PPGLs using exome sequencing and detected a postzygotic H3F3A mutation located on chromosome 1 in three tumors from one patient with a history of recurrent PPGL (13).

1.5 Newest germinal mutations involved in PPGLs

Comprehensive genetic analyses have identified germline SDHB and FH gene mutations as predominant causes of metastatic paraganglioma and pheochromocytoma.

Deletion of exon 1-3 in the SDHB gene is a novel germline mutation associated with hereditary HNPG (14).

However, a new research from 2018 shows a new germline mutation in the SLC25A11 gene, which encodes the mitochondrial 2-oxoglutarate/malate carrier and is responsible for PGLs with metastatic evolution (15).

In a case report of Olga Astapova *et al.* (16) from 2018, it has been presented a patient with VHL Syndrome-with bilateral pheochromocytoma who was found to have a heterozygous germline mutation, c.524A>G in the VHL gene, corresponding to the Y175C substitution in the protein. By comparing for the first time hypoxia response of Y175C-VHL cells lines with VHL-null 786-O cells line (derived from a human renal cell carcinoma), HIF2 α abundance was also similar in wild type and Y175C VHL-expressing cells after 6 hours and 12 hours of hypoxia. Also, these two variants of gene encode a VHL protein with the same response in normal oxygen condition. This mutation type was also described in another family with six patients (16).

In conclusion, we presented some of the latest advances in the rapidly evolving field of PPGLs and we focused on translational aspects from genetics to endocrinology. We should not forget that every patient is unique and every new, uncommon clinical and paraclinical feature can open a window to a new direction in science.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Cheung VKY, Gill AJ, Chou A. Old, New, and Emerging Immunohistochemical Markers in Pheochromocytoma and Paraganglioma. *Endocrine Pathol.* 2018; 29 (2):169–175.
2. Karasek D, Frysak Z, Pacak K. Genetic Testing for Pheochromocytoma. *Curr Hypertens Rep.* 2010; 12(6): 456–464.
3. Asban A, Kluijfhout WP, Drake FT, Beninato T, Wang E, Chomsky-Higgins K, Shen WT, Gosnell JE, Suh I, Duh QY. Trends of genetic screening in patients with pheochromocytoma and paraganglioma: 15-year experience in a high-volume tertiary referral center. *J Surg Oncol.* 2018;117(6):1217-1222.
4. Alrezk R, Suarez A, Tena I, Pacak K. Update of Pheochromocytoma Syndromes: Genetics, Biochemical Evaluation and Imaging. *Frontiers in Endocrinology.* 2018.
5. Janssen I, Blanchet EM, Adams K, Chen CC, Millo CM, Herscovitch P. Superiority of [68Ga]-DOTATATE PET/CT to other functional imaging modalities in the localization of SDHB-associated metastatic pheochromocytoma and paraganglioma. *Clin Cancer Res.* (2015) 21:3888–3895.
6. Strosberg J, Krenning E. 177Lu-dotatate for midgut neuroendocrine tumors. *N Engl J Med.* 2017; 376:1391–1392.
7. Gill AJ: Succinate dehydrogenase (SDH)-deficient neoplasia. *Histopathology.* 2018; 72:106–116.
8. Crespigio J, Berbel LCL, Dias MA, Berbel RF, Pereira SS, Pignatelli D, Mazzucco TL. Von Hippel–Lindau disease: a single gene, several hereditary Tumors. *Endocrinol Invest.* 2018; 41:21–31.
9. Evenepoel L, van Nederveen FH, Oudijk L, Papathomas TG, Restuccia DF, Belt EJT, de Herder WW, Feelders RA, Franssen GJH, Hamoir M, Maiter D, Ghayee HK, Shay JW, Perren A, Timmers HJLM, van ES, Vroonen L, Aydin S, Robledo M, Vikkula M, de Krijger RR, Dinjens WNM, Persu A, Korpershoek E. Expression of Contactin 4 Is Associated With Malignant Behavior in Pheochromocytomas and Paragangliomas. *J Clin Endocrinol Metab* 2018; 103(1):46-55.
10. Bazer WG, Cudd FW, Meininger TA, Spencer CJ. Maternal nutrition and fetal development. *J. Nutr.* 2004; 134: 2169-2172.
11. Wu TP, Wang T, Seetin MG, Lai Y, Zhu S. DNA methylation on N(6)- adenine in mammalian embryonic stem cells. *Nature.* 2016; 532: 329-333.
12. Job S, Draskovic I, Burnichon N, Buffet A, Cros J, Lepine C, Venisse A, Robidel E, Verkarre V, Meatchi T, Sibony M, Amar L, Bertherat J, de RA, Londono-Vallejo A, Favier J, Castro-Vega LJ, Gimenez-Roqueplo AP. Telomerase Activation and ATRX Mutations Are Independent Risk Factors for Metastatic Pheochromocytoma and Paraganglioma. *Clin Cancer Res.* 2019; 25(2):760-770.
13. Toledo RA, Qin Y, Cheng ZM, Gao Q, Iwata S, Silva GM, Prasad ML, Ocal IT, Rao S, Aronin N, Barontini M, Bruder J, Reddick RL, Chen Y, Aguiar RC, Dahia PL. Recurrent mutations of chromatin-remodeling genes and kinase receptors in pheochromocytomas and paragangliomas. *Clin Cancer Res.* 2016; 22:2301–2310.
14. de Vos B, Rijken JA, Adank MA, Hoksbergen AWJ, Bayley JP, Leemans CR, Hensen EF. A novel succinate dehydrogenase subunit B germline variant associated with head and neck paraganglioma in a Dutch kindred: A family-based study. *Clin Otolaryngol.* 2018; 43(3):841-845.
15. Buffet A, Morin A, Castro-Vega LJ, Habarou F, Lussey-Lepoutre C, Letouze E, Lefebvre H, Guilhem I, Haissaguerre M, Raingeard I, Padilla-Girola M, Tran T, Tchara L, Bertherat J, Amar L, Ottolenghi C, Burnichon N, Gimenez-Roqueplo AP, Favier J. Germline Mutations in the Mitochondrial 2-Oxoglutarate/Malate Carrier SLC25A11 Gene Confer a Predisposition to Metastatic Paragangliomas. *Cancer Res.* 2018; 78(8):1914-1922.
16. Astapova O, Biswas A, DiMauro A, Moalem J, Hammes SR. Bilateral Pheochromocytomas in a Patient with Y175C Von Hippel-Lindau Mutation. *Case Rep Endocrinol.* 2018;2018:8967159.