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Genotype and Tumor Locus Determine Expression Profile of Pseudohypoxic Pheochromocytomas and Paragangliomas[1,2]

Uma Shankavaram[*,3], Stephanie M. J. Fliedner[†,‡,3], Abdel G. Elkahloun[§], Jenifer J. Barb[¶], Peter J. Munson[¶], Thanh T. Huynh[†], Joey C. Matro[†], Hana Turkova[†], W. Marston Linehan[#], Henri J. Timmers[**], Arthur S. Tischler[††], James F. Powers[††], Ronald de Krijger[‡‡], Bora E. Baysal[§§], Martina Takacova[¶¶], Silvia Pastorekova[¶¶], David Gius[*,##], Hendrik Lehnert[‡], Kevin Camphausen[*] and Karel Pacak[†]

*Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD; [†]Section on Medical Neuroendocrinology, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; [‡]1st Department of Medicine, University Hospital of Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; [§]Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [¶]Mathematical and Statistical Computing Laboratory, Center for Information Technology, National Institutes of Health, Bethesda, MD; [#]Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD; **Department of Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [††]Department of Pathology, Tufts Medical Center, Boston, MA; [‡‡]Department of Pathology, Josephine Nefkens Institute, Erasmus MC-University Medical Center, Rotterdam, The Netherlands; [§§]Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY; [¶¶]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic; [##]Department of Radiation Oncology, Feinberg Northwestern Medical School, Chicago, IL

Abbreviations: AT, abdominal/thoracic; CSS, Carney-Stratakis Syndrome; HIF2α, hypoxia-inducible factor 2α; HN, head/neck; IPA, Ingenuity Pathway Analysis; NF1/*NF1*, neurofibromatosis 1 syndrome/gene; OXPHOS, oxidative phosphorylation; PAMR, prediction analysis for microarray; PGL, paraganglioma; PGL1, 2, 3, 4, familial PGL types 1, 2, 3, 4; PHD, prolyl hydroxylase; PHD2/EGLN1, prolyl hydroxylase 2; PHEO, pheochromocytoma; qRT-PCR, quantitative real-time polymerase chain reaction; SAM, significance analysis of microarray; SDH, succinate dehydrogenase; SDHA, SDH subunit A; SDHAF2, SDH complex assembly factor 2; SDHB, SDH subunit B; SDHC, SDH subunit C; SDHD, SDH subunit D; TMEM127, transmembrane protein 127; VHL/*VHL*, von Hippel-Lindau syndrome/gene

Address all correspondence to: Karel Pacak, MD, PhD, DSc, Chief, Section on Medical Neuroendocrinology, Professor of Medicine, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Building 10, CRC, 1-East, Room 1-3140, 10 Center Drive, Bethesda, MD 20892-1109. E-mail: karel@mail.nih.gov

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[3]These authors contributed equally.

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Abstract

Pheochromocytomas (PHEOs) and paragangliomas (PGLs) related to mutations in the mitochondrial succinate dehydrogenase (SDH) subunits A, B, C, and D, SDH complex assembly factor 2, and the von Hippel-Lindau (VHL) genes share a pseudohypoxic expression profile. However, genotype-specific differences in expression have been emerging. Development of effective new therapies for distinctive manifestations, e.g., a high rate of malignancy in SDHB- or predisposition to multifocal PGLs in SDHD patients, mandates improved stratification. To identify mutation/location-related characteristics among pseudohypoxic PHEOs/PGLs, we used comprehensive microarray profiling (SDHB: $n = 18$, SDHD-abdominal/thoracic (AT): $n = 6$, SDHD-head/neck (HN): $n = 8$, VHL: $n = 13$). To avoid location-specific bias, typical adrenal medulla genes were derived from matched normal medullas and cortices ($n = 8$) for data normalization. Unsupervised analysis identified two dominant clusters, separating SDHB and SDHD-AT PHEOs/PGLs (cluster A) from VHL PHEOs and SDHD-HN PGLs (cluster B). Supervised analysis yielded 6937 highly predictive genes (misclassification error rate of 0.175). Enrichment analysis revealed that energy metabolism and inflammation/fibrosis-related genes were most pronouncedly changed in clusters A and B, respectively. A minimum subset of 40 classifiers was validated by quantitative real-time polymerase chain reaction (quantitative real-time polymerase chain reaction *vs.* microarray: $r = 0.87$). Expression of several individual classifiers was identified as characteristic for VHL and SDHD-HN PHEOs and PGLs. In the present study, we show for the first time that SDHD-HN PGLs share more features with VHL PHEOs than with SDHD-AT PGLs. The presented data suggest novel subclassification of pseudohypoxic PHEOs/PGLs and implies cluster-specific pathogenic mechanisms and treatment strategies.

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Introduction

Predispositions to certain tumors have been linked to an ever-increasing number of mutations. To date, mutations in 11 different genes have been associated with development of paragangliomas (PGLs), which are catecholamine-producing, chromaffin cell tumors, including adrenal pheochromocytomas (PHEOs). Initially, discovery of mutations was guided by syndromic presentation and family history; however, more recently discovered mutations can present in seemingly sporadic fashion. Known PHEO/PGL susceptibility genes are *von Hippel-Lindau* (*VHL*) and *neurofibromatosis 1* (*NF1*) in the homonymous syndromes (VHL and NF1, respectively), *RET proto-oncogene* in multiple endocrine neoplasia type 2, *succinate dehydrogenase D* (*SDHD*) in familial PGL type 1 (PGL1) and Carney-Stratakis Syndrome (CSS), *SDHC* in PGL3 and CSS, *SDHB* in PGL4 and CSS, *SDH complex assembly factor 2* (*SDHAF2*) in PGL2, *prolyl hydroxylase 2* (*PHD2/EGLN1*), *transmembrane protein 127* (*TMEM127*), *kinesin family member 1B*, *SDHA*, and *MYC-associated factor X* (reviewed in [1]). Most recently, activating mutations in hypoxia-inducible factor 2α (*HIF2*α) have been associated with PGL and polycythemia [2].

Notwithstanding the multitude of susceptibility genes, mutation-derived PHEOs/PGLs separate into merely two main clusters, one containing *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, and *VHL* and the other consisting of *NF1*, *RET proto-oncogene*, *TMEM127*, *kinesin family member 1B*, and *MYC-associated factor X* mutation–derived PHEOs/PGLs (reviewed in [1,3]). The PHEOs/PGLs of the first mentioned cluster are characterized by a pseudohypoxic phenotype, i.e., inappropriate stabilization of HIFα subunits under normoxia (reviewed in [1,3]).

Under normoxia, hydroxylation of HIFα by PHDs (PHD1/EGLN2, PHD2/EGLN1, and PHD3/EGLN3) designates them for VHL-dependent ubiquitylation and subsequent degradation [4]. Accordingly, *VHL* mutations can promote HIFα stabilization. Similarly, SDH dysfunction causes HIF stabilization by succinate or reactive oxygen species accumulation-mediated PHD inhibition [5,6].

Despite increasing evidence for differences within the pseudohypoxic cluster [7–9], the molecular basis for distinct clinical behaviors including the preferential site of tumor development, biochemical phenotype, or metastatic potential remains largely unknown. Mutations in *SDHB* have been associated with extra-adrenal PGLs and high risk of malignancy [1,3]. *SDHD* mutations, however, predispose to multifocal PHEOs/PGLs, primarily from the head and neck (HN) region, with low metastatic risk [3]. However, HN PGL can be inoperable, or surgery can lead to severe side effects due to close vicinity to major blood vessels and nerves. *VHL* mutation–derived PHEOs/PGLs are almost always adrenal, non-metastatic, but frequently bilateral and/or recurrent, thus adrenal sparing treatment options are of high importance [3]. SDHA, SDHC, and SDHAF2 mutation–derived PHEOs/PGLs are rare and have not yet been characterized in detail. Large cohort studies including PHEO/PGL patients revealed that SDHA, SDHC, and SDHAF2 are extremely rare (0.1–0.5%, 0.4–2.2%, and non-detectable, respectively) [9–13]. In a Dutch population of HN PGL patients, SDHAF mutations were found in 4% and SDHC mutations in 0.4% of cases (SDHA not tested) [14].

To date, genetic testing presents an important diagnostic tool for risk assessment of PHEO/PGL patients and their families. However, genetic testing is cost intensive and targeted therapeutic options for