Paraganglioma of the organ of Zuckerkandl associated with a somatic HIF2α mutation: A case report

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Abstract. Paragangliomas of the organ of Zuckerkandl (OZ-PGL) are rare tumors that, in >70% of cases, occur in association with succinate dehydrogenase complex iron sulfur subunit B (SDHB) or SDHD gene mutations. The aim of the current study was to determine whether a somatic genetic defect in the hypoxia-inducible factor 2α (HIF2α) gene was present in a case of sporadic OZ-PGL. A 32-year-old African female presented with uncontrolled hypertension during the first trimester of pregnancy. A diagnostic hysteroscopy was performed 3 months after delivery, precipitating a hypertensive crisis. Thereafter, the patient was diagnosed with noradrenaline-secreting OZ-PGL. A complete blood count showed impaired HIF2α/β-glucose levels. To the best of our knowledge, the present case represents the first of its kind to associate a somatic HIF2α gain-of-function mutation with OZ-PGL. It is therefore recommended that patients without germline SDHx mutations should be tested for HIF2α mutations.

Introduction

In 1901, Emile Zuckerkandl first described the abdominal para-aortic paraganglia in fetal and newborn humans as a paired retroperitoneal organ located laterally to the abdominal aorta at the level of the inferior mesenteric aorta (1). This paraganglionic complex, known as the organ of Zuckerkandl (OZ), also includes smaller accessory paraganglia located anteriorly to the aorta between the lateral organs or below the aortic bifurcation (2). In 1903, Alfred Kohn established that the OZ commonly originated from chromaffin cells of the adrenal medulla (3), and it has later been established that it constitutes the largest accumulation of extradrenal chromaffin cells in mammals. In humans, the OZ reaches its maximal size at
the age of ~3 years and subsequently regresses after reaching its peak by autophagy (4). The OZ is considered to be most important physiologically throughout the early gestational period, during which it secretes catecholamines into the fetal circulation, functioning as a homeostatic regulator of blood pressure (5). The OZ represents a site of origin for paragangliomas (PGLs) that preferentially secrete norepinephrine and induce symptoms of catecholamine excess (6). OZ-PGLs are rare tumors typically located close to the origin of the inferior mesenteric artery or between the proximal common iliac arteries (1). These lesions may occur sporadically or, in ~70% of cases, in association with succinate dehydrogenase complex iron sulfur subunit B (SDHB) or, less commonly, SDHD gene mutations (7). In addition, OZ-PGLs are particularly aggressive with high rates of metastatic spread (8). At least 150 cases of OZ-PGLs have been reported in the literature. They are strongly associated with an aggressive behavior, likely associated with the SDHB mutation status (7). Due to the rarity of this disease, not much is known about its natural history. A single-center retrospective study of 371 patients with either pheochromocytoma or sympathetic paraganglioma revealed only 21 cases of OZ-PGLs, 14 of which (66%) had metastases at diagnosis (9). To the best of our knowledge, the current case demonstrates that somatic HIF2α [also known as endothelial PAS domain-containing protein 1 (EPAS1)] mutations may be associated with OZ-PGL for the first time.

Case report

In September 2014, a 32-year-old African woman native to Burkina Faso was referred to the hypertension unit of La Timone University Hospital (Marseille, France) for screening for secondary hypertension. Hypertension was initially noted during the first trimester of pregnancy. The patient went into premature labor at 22 weeks and a cesarean delivery was performed 15 days later; the baby did not survive and succumbed a few minutes after birth. Following delivery, the patient experienced persistent and uncontrolled hypertension despite taking nicardipine (60 mg/day) and labetalol (400 mg/day) for 3 months. A diagnostic hysteroscopy was performed 3 months later, precipitating a hypertensive crisis [systolic blood pressure (BP), 300 mmHg; normal, <140 mmHg]. Thereafter, the patient was referred to the hypertension unit of La Timone University Hospital for secondary hypertension screening in September 2014. There was no known family history of tumors, syncope or sudden death. At admission (weight, 51 kg; height, 163 cm; and body mass index, 19.2), the patient presented with headaches, recurring episodes of palpitations and sweating, chest tightness, and polyuria. Treatment with nicardipine and labetalol was replaced with verapamil (240 mg/day). Ambulatory 24-h BP monitoring was performed during treatment with verapamil and demonstrated that the patient maintained a BP of 155/96 mmHg. Prazosin (2.5 mg once per day) was subsequently administered to reduce blood pressure further until surgical intervention.

Additional laboratory tests identified elevated 24 h urinary normetanephrine levels [20,140 nmol/24 h; upper reference limit (URL), <1900 nmol/24 h] and normal metanephrine levels (380 nmol/24 h; URL, <1600 nmol/24 h). In addition, serum chromogranin A was observed to be elevated (223 μg/l; URL, <100 μg/l). A complete blood count revealed mild normocytic anemia (hemoglobin count, 108.0 g/l; normal hemoglobin count for female adults, 11.5-15.0 g/dl; mean corpuscular volume, 83.4 fl; normal mean corpuscular volume of adults, 80-100 fl) of an inflammatory origin with normal platelets and leukocytes. Diagnostic computed tomography (CT) revealed a 40-mm hypervascular, heterogeneous, left para-aortic mass located at the level of the inferior mesenteric artery (Fig. 1A). 18F-FDG PET/CT confirmed the diagnosis of OZ-PGL without multifocal disease. The tumor also exhibited moderate heterogeneous 18F-fluorodeoxyglucose uptake (Fig. 1B). In October 2014, complete surgical resection was performed. Histopathological analysis of the tumor tissue revealed typical PGL features, including a low Ki-67 index (<1%) (monoclonal mouse antibody; clone, MIB-1; catalogue no., M7240; dilution, 1:100: Dako, Glostrup, Denmark). Genetic testing for germline mutations (including large deletions) in the von Hippel-Lindau tumor suppressor (VHL), succinate dehydrogenase complex iron sulfur subunit B (SDHB), SDHC

Figure 1. Imaging and pathological features of the OZ-PGL. (A) Contrast-enhanced CT (arterial phase) showing a 40-mm hypervascular and heterogeneous left para-aortic mass located at the level of the IMA (asterisk). (B) 18F-FDOPA PET/CT (lower image) showing a single tumor. (C) Iodine-123-metaiodobenzylguanidine scintigraphy also positively located the mass (planar anterior view). (D) Immunohistochemical analysis of the tumor demonstrated positive glucose transporter-1 immunostaining (~10%). CT, computed tomography; IMA, inferior mesenteric artery; 18F-FDOPA, 18F-fluorine-L-dihydroxyphenylalanine; 18F-FDG, 18F-fluorodeoxyglucose; PET, positron emission tomography.
and SDHD genes was normal. Immunostaining demonstrated that the tumor cells were positive for SDHB. Further genetic testing revealed a heterozygous cysteine to tyrosine substitution at base 1589 (c.1589Cys>Tyr) in the HIF2α coding sequence of the OZ-PGL, resulting in the replacement of alanine with valine at amino acid position 530 (Ala530Val). This leads to HIF2α stabilization as described by a previous in vitro experiment (10). A germline HIF2α mutation was excluded based on the negative results of blood DNA testing.

In order to assess the metabolic properties of the tumor, the present study performed 1H-high-resolution magic-angle-spinning (HRMAS) nuclear magnetic resonance spectroscopy-based global metabolomic profiling on tumor samples. A one-dimensional proton spectrum (1.5-7.2 ppm) using a Carr-Purcell-Meiboom-Gill pulse sequence with water presaturation was acquired from each intact tissue sample. Low levels of succinate were detected, and according to our previous study (11) this excludes a SDH deficiency. Notably, the tumor also exhibited abnormally high levels of α- and β-glucose isomers as identified by HRMAS (Fig. 2). The patient is currently in remission, and regular clinical follow up occurred every 6 months with normal metanephrines.

Discussion

To the best of our knowledge, the present case demonstrates, for the first time, that patients with somatic HIF2α mutations may present with OZ-PGL.

Germline mutations in the HIF2α/EPAS1 gene have been previously associated with congenital polycythemia (12). A syndromic association has been reported between somatic gain-of-function mutations in HIF2α and congenital imaging (PET-FDOPA) at 1 year post-intervention were also normal.

Written informed consent was obtained from the patient for publication of the present case report and any accompanying images.
polycythemia, multiple PGL, duodenal somatostatinoma and ocular vascular abnormalities (for example, Pacak-Zhuang syndrome) (10,13-16). Mutations in HIF2α have also been observed in apparently sporadic pheochromocytomas (PHEOs)/PGLs without polycythemia (17-19). In one study, mutations (exon 12) were identified in 2 cases of solitary PHEO and 1 para-adrenal PGL (18). In an additional study, 6/42 cases of apparently sporadic PHEOs were identified to have HIF2α mutations (3 in exon 9 and 3 in exon 12) (17).

HIF2α protein stability is dependent on the hydroxylation of two specific proline residues (Pro405 and Pro531) located in the O2-dependent degradation domain (10). Until present, all mutations described were known to be located in hot spots adjacent to hydroxylation sites (16). These specific mutations disturb HIF2α prolyl hydroxylation and subsequent recognition by the VHL protein, resulting in the failure of HIF2α degradation via ubiquitination (16). As mutant HIF2α protein has a longer half-life compared with the wild-type protein, it has a targeted effect downstream of HIF2α (10).

The mutation identified in the present study had previously been reported in a case of apparently sporadic PHEO/PGL (18). The mutation involved Ala530, which is located in close proximity to the second hydroxylation site (Pro531) and at the interface with VHL and EGL-9 family hypoxia-inducible factor 1 (EGLN1) client-proteins. Homology modeling was performed to outline the biological properties of the Val530 mutant (Fig. 3). These three-dimensional models were generated with IBM SPSS Modeler v14 (IBM SPSS, Armonk, NY, USA) using the crystal structures of HIF1α in interaction with EGLN1 or VHL as templates. HIF1α and HIF2α exhibit a sequence identity of 65% in the region modeled, which guarantees (>50% identity) that the models are of a high quality. The model anticipates that valine, a larger residue than alanine, increases steric hindrance at Pro531, leading to: i) A reduction in its accessibility to EGLN1 by inhibition of Pro531 hydroxylation; and ii) impairment of HIF2α/VHL interaction with decreased HIF2α ubiquitination. The present study also identified a novel metabolic pattern with low succinate and high glucose levels associated with HIF2α mutation. Abnormally high levels of glucose may be explained by increased glucose uptake induced by HIF2α stabilization (20).

In conclusion, to the best of our knowledge, the current study identified, for the first time, an association between somatic HIF2α mutations and OZ-PGL. It is therefore recommended that patients with OZ-PGL in the absence of germline SDHx mutations should undergo testing for HIF2α mutations.

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