Abstract:
A 53-year-old woman was admitted to a hospital for gradual left-ear hearing loss over 2 years. Head computed tomography revealed a 2-cm mass along the left jugular bulb and another at the right carotid bulb. The right tumor was resected; the pathological diagnosis was carotid body paraganglioma. Mutations of succinate dehydrogenase (SDH) were suspected, but SDHB staining remained in the tumor. Genetic testing identified a known SDHB mutation (L157X). The patient had head and neck paraganglioma with an SDHB mutation (L157X) more typical of an SDHD mutation. SDHB immunohistochemistry is useful for detecting SDHx mutations, but careful interpretation is needed.

Key words: paraganglioma, head and neck paraganglioma, succinate dehydrogenase, L157X, positron emission tomography, brown adipose tissue

Case Report

A 53-year-old woman was admitted to a nearby hospital for gradual left-ear hearing loss over a 2-year period. The patient had no headaches or hyperhidrosis. She had no notable medical history except that her mother had hypertension. Her body mass index (BMI) was 18.3 kg/m², and her blood pressure (BP) was 118/72 mmHg. Blood tests showed mild hyperlipidemia with no abnormalities of glucose metabolism; fasting plasma glucose was 103 mg/dL, HbA1c (NGSP) was 6.1%, total cholesterol was 247 mg/dL, and tri...
Figure 1. Gadolinium-enhanced MRI of the head shows an approximately 2-cm enhanced mass along the left jugular bulb and another on the right carotid bulb (A, B). Yellow triangles show the location of the tumors. 18F-FDG PET/CT shows the focal uptake of FDG in the left-side head tumor (SUVmax 23.5) and a multifocal uptake of FDG in the mediastinum (E-H). 18F-FDG PET/CT findings were compared with those of contrast-enhanced CT and MRI of the chest; no masses matched the FDG uptake, suggesting a physiological uptake in brown adipose tissue (G-J). Hematoxylin and Eosin staining of the right carotid bulb tumor shows the proliferation of tumor cells with a Zellballen pattern (C). The tumor cells are positive for chromogranin A (D). Immunohistochemistry revealed persistent SDHB staining with weak-diffuse pattern (K). C: ×400 magnification, D: ×200 magnification, and K: ×100 magnification. 18F-FDG PET/CT: 18F-fluorodeoxyglucose positron emission tomography/computed tomography.

Glycerides were 80 mg/dL.

Audiometry revealed hearing loss in the left ear, but otoscopy showed no abnormalities of the auditory canal or the tympanic membrane. The presence of an intracranial tumor was therefore suspected, and imaging studies of the head were performed. Computed tomography (CT) and magnetic resonance imaging (MRI) of the head revealed an approximately 2-cm mass along the left jugular bulb and another at the right carotid bulb (Fig. 1A, B). Adrenal hormone levels were measured as follows: adrenaline 63 ng/mL (reference: ≤100), noradrenaline 2,199 ng/mL (reference: 100-450), and dopamine 0.053 ng/mL (reference: ≤0.20).

The right tumor was completely resected; the pathological diagnosis was a right carotid body paraganglioma (Fig. 1C, D). The left tumor was targeted with gamma knife radiotherapy with a dose of 14 Gy, with consideration of the risk of inner-ear dysfunction due to surgery. Six months after surgery, the patient underwent 18F-fluorodeoxyglucose-positron emission tomography/CT (18F-FDG PET/CT) in the previous hospital; the image showed the multifocal uptake of FDG in the supraclavicular, paraaortic, and paravertebral areas (Fig. 1E-H). The patient was therefore referred to us for the development of therapeutic strategies for HNPGL.

On admission to our hospital, her blood pressure (BP) was 147/84 mmHg. Her ambulatory BP was measured over a full day; the mean BP was 131/74 mmHg, and the max BP was 178/92 mmHg. Adrenal hormone tests were reevaluated in our hospital: adrenaline 0.05 ng/mL (reference: 0-0.10), noradrenaline 1.59 ng/mL (reference: 0.1-0.5), dopamine 0.04 ng/mL (reference: 0.1-0.03), urinary-
Multiple mediastinal paragangliomas or lymph node metastases were initially suspected due to the 18F-FDG PET/CT findings, but no apparent masses or paraganglioma lesions were detected by contrast-enhanced CT or magnetic resonance imaging (MRI) (Fig. 1G-J). No uptake in the multifocal lesions was shown by 123I-metaiodobenzylguanidine (MIBG) scintigraphy. Comparing imaging studies, the multifocal FDG uptake was thought to have been a physiological uptake by brown adipose tissue.

The patient started doxazosin 1.0 mg/day for hypertension. She experienced presyncope due to a decrease in her blood pressure, so the dose of doxazosin was reduced to 0.5 mg/day. Six months later, as an outpatient, she showed no symptoms of catecholamine excess, such as headache, hyperhidrosis or constipation, and her BP was well-controlled.

The adrenal hormone levels and excretion of metabolites were relatively unchanged: adrenaline 0.04 ng/mL, noradrenaline 1.49 ng/mL, dopamine 0.04 ng/mL, urinary-metanephrine 0.11 mg/day, and urinary-normetanephrine 0.90 mg/day.

Based on the tumor locations and pathological diagnosis of carotid paraganglioma, an SDHD mutation was suspected. SDHB immunostaining of the tumor was performed to detect the absence of SDHB staining due to SDHx mutations, but SDHB was actually immunopositive with a weak-diffuse pattern (Fig. 1K). We performed further genetic testing to confirm the presence of an SDHx mutation and to assess the risk of metastasis. Genomic DNA was extracted from peripheral blood leukocytes and a paraffin-embedded section of the tumor. The coding exons and adjacent intron sequences of SDHB and SDHD were amplified by polymerase chain reaction followed by Sanger sequencing using appropriate primers. Surprisingly, genetic testing from a peripheral blood sample identified a known SDHB germline mutation (L157X) (Fig. 2, upper). No SDHD germline mutations were identified (data not shown). A sequence analysis of the tumor genomic DNA identified the corresponding SDHB somatic mutation (Fig. 2, lower), or LOH.

**Discussion**

According to recent reports, over 30% of patients with PPGL carry germline mutations, which is more frequent than the rates described in older reports (2). Succinate dehydrogenase (SDH) is located in the inner mitochondrial membrane and participates in the citric acid cycle and electron transport chain. Mutations of SDHs promote hypoxic signal transduction, which leads to tumorigenesis (1). Genotype-phenotype correlations associated with SDH mutations have been previously reported (1-4). The SDHB mutation is asso-

**(Figure 2. Results of a Sanger sequence analysis of SDHB exon 5. The DNA sequence isolated from peripheral white blood cells (germline) shows c.470delT, resulting in a change from a leucine to a stop codon at position 157 (L157X). Note the relative loss of the wild-type sequence signal in the tumor (somatic) compared to peripheral white blood cells (germline), or loss of heterozygosity (LOH). Wild: wild-type sequence of SDHB exon 5, Mutation: L157X mutation sequence of SDHB exon 5. Germline: germline mutation, Somatic: somatic mutation)**

| CCT | TAT | TTG>TGA (c.470delT) |
|-----|-----|---------------------|
| 155 | 156 | 157                 |
ciated with abdominal extra-renal lesions and metastatic disease (6), while the SDHD mutation is associated with head and neck lesions that are unlikely to cause metastatic disease (8).

The present patient initially had manifestation of head and neck tumors but was carrying an SDHB mutation. Genetic testing showed c.470delT in exon 5 of SDHB, resulting in a change from a leucine to a stop codon at position 157 (L157X). The same germline mutation in two patients with PPGL has been previously reported (9, 10). According to the reports, all of these patients harboring the SDHB (L157X) mutation had manifestations of paraaortic tumors, in contrast to our patient, who had head and neck tumors. One of the previous patients showed multiple metastases to the lungs, a typical malignant feature of SDHB mutation. Our patient, on the other hand, was characterized by manifestations mimicking PPGL with an SDHD mutation rather than SDHB mutations.

HNPGL is derived from neural crest cells and does not generally secrete catecholamine, developing along parasympathetic nerves (11). In association with SDH mutations, approximately 80-98% of SDHD mutation carriers have head and neck lesions, while 15-31% of SDHB mutation carriers also have HNPGL (3, 8, 12). HNPGL patients carrying SDHB germline mutations were recently evaluated, and the metastatic rate was found to be only 6% (11). This clinical feature differed from the findings of a previous report on SDHB mutation carriers without head and neck lesions (6).

Our patient also showed indolent clinical features with no occurrence of metastatic lesions or enlargement of the pre-existing tumors during one year of follow-up.

18F-FDG PET/CT is reportedly useful in some cases of PPGL, not showing an 123I-MIBG uptake on scintigraphy. Excess catecholamine secretion activates brown adipose tissues in humans, which can result in a multifocal FDG uptake in PPGL (13). The brown adipose tissue is distributed across the supraclavicular, paraaortic, and paravertebral areas, as was seen in our case. The careful interpretation and assessment of the meaning of the uptake are therefore required when 18F-FDG PET/CT imaging is used for an evaluation.

SDHB immunostaining is useful for detecting the presence of an SDHx mutation in HPPS. SDHB immunohistochemistry typically shows granular cytoplasmic staining (mitochondrial pattern) in non-SDHx-mutated tumors, whereas SDHx-mutated tumors are not stained with SDHB. Loss of SDH enzymatic activity suggests the biallelic inactivation of SDH genes (6). The sensitivity and specificity are reportedly high, so SDHB immunostaining is recommended when considering the indication of SDHx germline mutation testing (5, 6). The staining result, however, should be interpreted with extreme caution. SDHB immunostaining of some SDHx-mutated tumors reportedly demonstrates a weak-diffuse pattern, characterized by very mild cytoplasmic or nuclear blush (7, 14, 15). The weak-diffuse pattern is more frequent in tumors with SDHD mutations than in those with SDHB mutations. It has therefore been speculated that a nonfunctional misfolded SDH complex may result in abnormal staining patterns (15). The present case demonstrated a weak-diffuse pattern on SDHB immunostaining, and genetic testing ultimately identified a known SDHB mutation. SDHB immunostaining was not confirmed in the two previously reported cases with SDHB mutations (L157X) (9, 10).

In our patient, hypertension was well-controlled with a low dose of alpha blockade, and there were no catecholamine hypersecretion-associated symptoms. We therefore decided to adopt the careful wait-and-see follow-up approach reserved for asymptomatic patients with PPGL (16). The patient is now being closely monitored with measurement of the urinary-noradrenaline levels and multimodality imaging studies (17).

Conclusion

We herein report rare case of HNPGL with an SDHB mutation (L157X), which are typical phenotypes of an SDHD mutation. SDHB immunohistochemistry is a valuable tool for detecting SDHx mutations, but careful interpretation is needed.

All procedures reported here were in accordance with the ethical standards of the Wakayama Medical University Hospital Ethics Committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

The authors state that they have no Conflict of Interest (COI).

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References

1. Galan SR, Kann PH. Genetics and molecular pathogenesis of pheochromocytoma and paraganglioma. Clin Endocrinol (Oxf) 78: 165-175, 2013.
2. Lenders JW, Duh QY, Eisenhofer G, et al. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 99: 1915-1942, 2014.
3. Neumann HP, Pawlu C, Peczokska M, et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. JAMA 292: 943-951, 2004.
4. Andrews KA, Ascher DB, Pires DEV, et al. Tumour risks and genotype-phenotype correlations associated with germline variants in succinate dehydrogenase subunit genes SDHB, SDHC and SDHD. J Med Genet 55: 384-394, 2018.
5. Van Nederveen FH, Gaal J, Favier J, et al. An immunohistochemical procedure to detect patients with paraganglioma and pheochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol 10: 764-771, 2009.
6. Gimenez-Roqueplo AP, Favier J, Rustin P, et al. Mutations in the SDHB gene are associated with extra-adrenal and/or malignant pheochromocytomas. Cancer Res 63: 5615-5621, 2003.

7. Papathomas TG, Oudijk L, Persu A, et al. SDHB/SDHA immunohistochemistry in pheochromocytomas and paragangliomas: a multicenter interobserver variation analysis using virtual microscopy: a Multinational Study of the European Network for the Study of Adrenal Tumors (ENS@T). Mod Pathol 28: 807-821, 2015.

8. Benn DE, Gimenez-Roqueplo AP, Reilly JR, et al. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab 91: 827-836, 2006.

9. Saito T, Saito Y, Matsumura K, et al. Novel mutation (L157X) in the succinate dehydrogenase B gene (SDHB) in a Japanese family with abdominal paraganglioma following lung metastasis. Endocr J 56: 451-458, 2009.

10. Saito H, Kanai G, Hirabayshi K, Kajiwara H, Isahara Y, Osamura RY. L157X nonsense mutation of the succinate dehydrogenase subunit B gene in a Japanese patient with right paraaortic paraganglioma. Endocrine 38: 18-23, 2010.

11. Rijken JA, Niemeijer ND, Leemans CR, et al. Nationwide study of patients with head and neck paragangliomas carrying SDHB germline mutations. BJS Open 2: 62-69, 2018.

12. Williams MD. Paragangliomas of the head and neck: an overview from diagnosis to genetics. Head Neck Pathol 11: 278-287, 2017.

13. Hadi M, Chen CC, Whatley M, Pacak K, Carraquillo JA. Brown fat imaging with "F-6-fluorodopamine PET/CT, "F-FDG PET/CT, and "I-MIBG SPECT: a study of patients being evaluated for pheochromocytoma. J Nucl Med 48: 1077-1083, 2007.

14. Gill AJ, Benn DE, Chou A, et al. Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. Hum Pathol 41: 805-814, 2010.

15. Castelblanco E, Santacana M, Valls J, et al. Usefulness of negative and weak-diffuse pattern of SDHB immunostaining in assessment of SDH mutations in paragangliomas and pheochromocytomas. Endocr Pathol 24: 199-205, 2013.

16. Hescot S, Leboulleux S, Amar L, et al. One-year progression-free survival of therapy-naive patients with malignant pheochromocytoma and paraganglioma. J Clin Endocrinol Metab 98: 4006-4012, 2013.

17. Plouin PF, Amar L, Dekkers OM, 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.