【 CASE REPORT 】

Head and Neck Paraganglioma Atypically Carrying a *Succinate Dehydrogenase Subunit B* Mutation (L157X)

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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 (L 157X) 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

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Introduction

Pheochromocytoma and paraganglioma (PPGL) are neuroendocrine tumors originating from the adrenal medulla and paraganglia. Genetic testing shows that over 30% of patients with PPGL carry germline mutations (1, 2). *Succinate dehydrogenase subunit B* (*SDHB*) and *D* (*SDHD*) are genes responsible for hereditary pheochromocytoma-paraganglioma syndrome (HPPS); *SDHB* is predisposed to metastatic disease, while *SDHD* is associated with multifocal head and neck paragangliomas (HNPGLs) (3). Genetic testing of patients with PPGL is beneficial for the personalized management of the proband and can allow for the early diagnosis and treatment of their relatives (4).

Immunohistochemistry for SDHB is reportedly a valuable tool for detecting patients with PPGL carrying *SDHA*, *SDHB*, *SDHC*, or *SDHD* mutations (5). Loss of normal allele [loss of heterozygosity (LOH)] in tumor DNA leads to complete loss of the SDHB function, resulting in the ab-

sence of SDHB immunostaining (6). Careful interpretation of the results is needed, so a previous report considered the reproducibility of the assessment methods (7).

In the present case, an *SDHB* germline mutation (L157X) carrier presented with HNPGL, which is generally typical of patients harboring *SDHD* mutations. The interpretation of the immunohistochemistry was difficult, however, because SDHB immunostaining remained in the tumor.

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/m^2, 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-
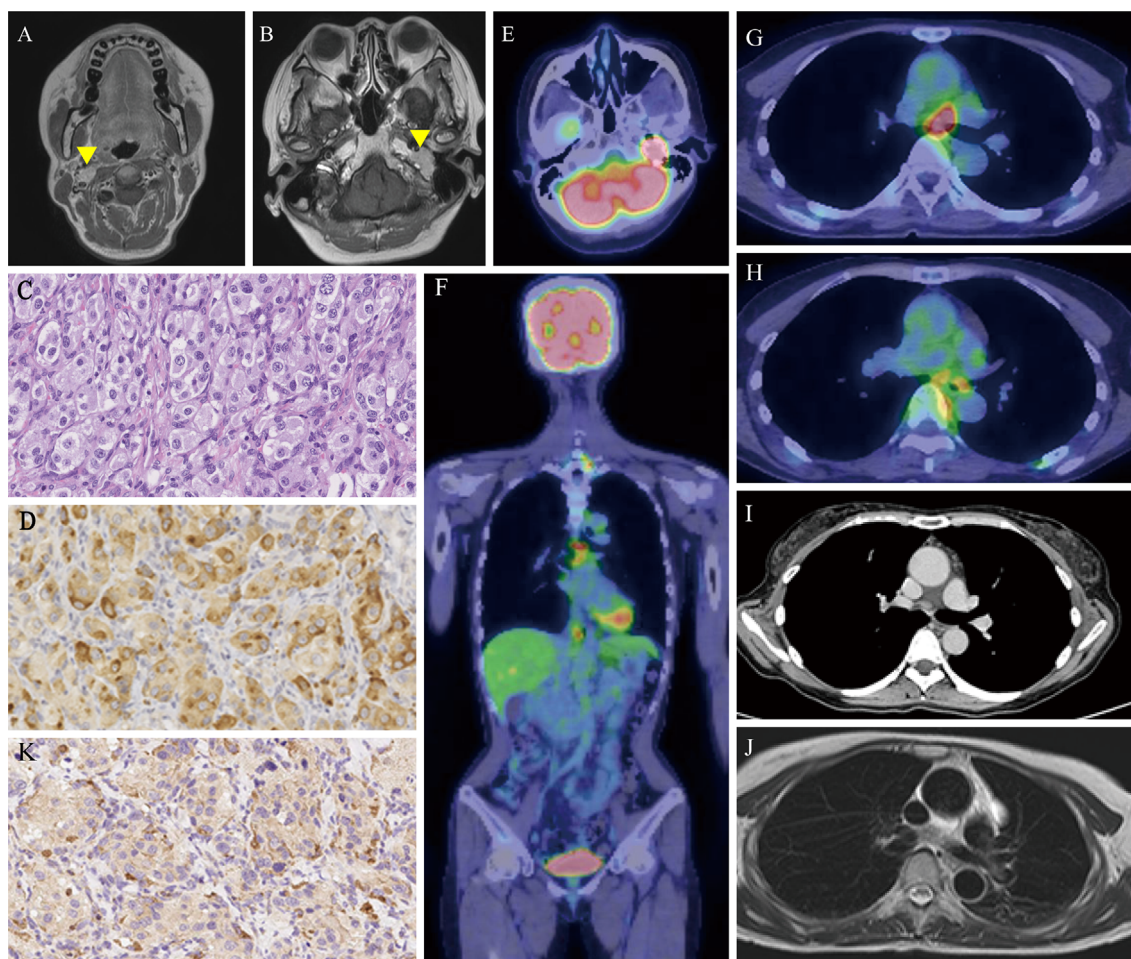
Figure 1. Gadolinium-enhanced MRI of the head shows an approximately 2-cm enhanced mass along the left jugular bulb and another on the right carotid bulb (A, B). Yellow triangles show the location of the tumors. ^{18}F-FDG PET/CT shows the focal uptake of FDG in the left-side head tumor (SUV$_{max}$ 23.5) and a multifocal uptake of FDG in the mediastinum (E-H). ^{18}F-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. ^{18}F-FDG PET/CT: ^{18}F-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 ^{18}F-fluorodeoxyglucose-positron emission tomography/CT (^{18}F-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-
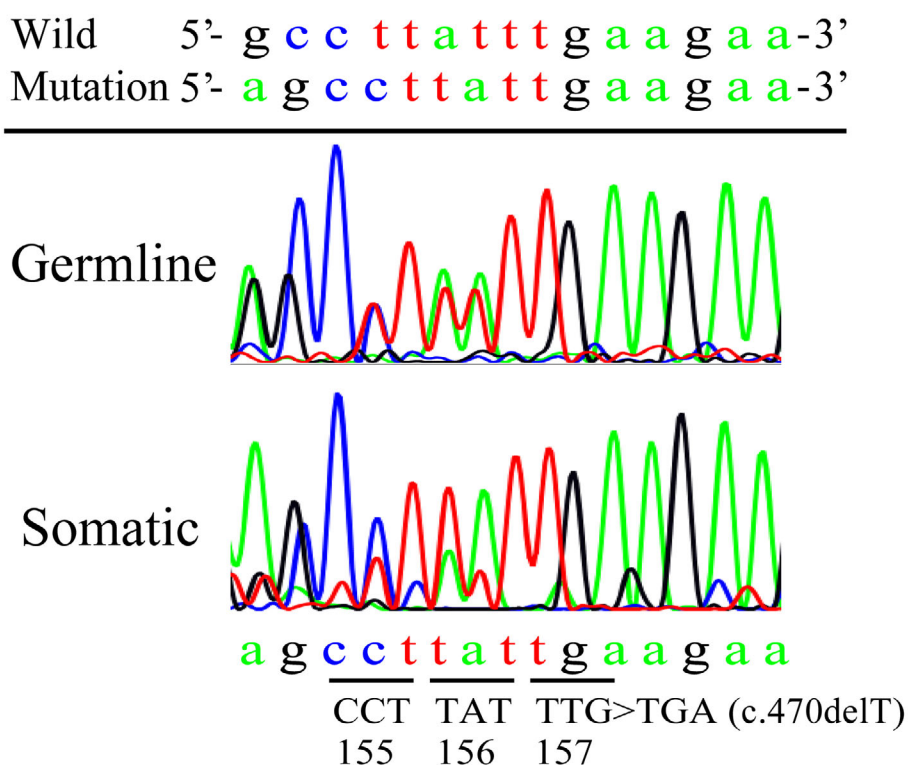
Wild   5'- g c c t t a t t t g a a g a a -3'
Mutation 5'- a g c c t t a t t g a a g a a -3'

Germline

Somatic

a g c c t t a t t g a a g a a
CCT TAT TTG>TGA (c.470delT)
155 156 157

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**

metanephrine 0.10 mg/day (reference: 0.04-0.18), and urinary-normetanephrine 0.81 mg/day (reference: 0.1-0.28).

Multiple mediastinal paragangliomas or lymph node metastases were initially suspected due to the ^{18}F-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 ^{123}I-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 *SDH*s 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-

ciated with abdominal extra-adrenal 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 (L157 X). 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.

^{18}F-FDG PET/CT is reportedly useful in some cases of PPGL not showing an ^{123}I-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 ^{18}F-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-normetanephrine 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.

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