Positive Immunostaining for Succinate Dehydrogenase B (SDHB) in Paraganglioma Associated with Germline Mutation of SDHB, L157X and P236S

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Purpose: Pheochromocytoma (PCC) and paraganglioma (PGL) associated with the succinate dehydrogenase (SDH) germline mutations are characterized by negative results of immunohistochemistry tests for SDH subunit B (SDHB). Genetic testing for the SDH complex (SDHA, SDHB, SDHC, SDHD, and SDHAF2) is indicated only in patients with those diseases in whom immunohistochemistry tests for SDHB as a surrogate marker to detect the SDH complex mutation yield negative results. Two novel SDHB germline mutations, L157X and P236S, in PGL were previously reported. We therefore examined immunohistochemistry testing for SDHB in the PGLs with the SDHB germline mutations of L157X and P236S.
Methods: Immunohistochemistry for SDHB was performed in PGLs with the SDHB germline mutations of L157X and P236S. Five cases of sporadic PCC were subject to immunohistochemistry testing for SDHB. Normal tissue from the adrenal cortex adjacent to the sporadic PCC was used as the external positive control.
Results: Immunohistochemistry results were positive for SDHB in PGLs with the SDHB germline mutation of L157X and P236S, all five cases of sporadic PCC, and the adrenal cortex as the external positive control.
Conclusion: Immunohistochemistry tests for SDHB showed positivity in PGLs associated with the SDHB germline mutations of L157X and P236S. Thus, immunohistochemistry testing for SDHB might not always reveal a surrogate marker in formal genetic testing of the SDH complex.

Key words: Paraganglioma, SDHB mutation, Immunohistochemistry

INTRODUCTION

Pheochromocytoma (PCC) and Paraganglioma (PGL) are neuroendocrine tumors arising from the adrenal medulla and the sympathetic or parasympathetic paraganglia, respectively [1, 2]. As defined by the World Health Organization, PCC is also an intra-adrenal PGL that arises from the chromaffin cells of the adrenal medulla [2]. PCC and PGL can occur sporadically or as part of different hereditary tumor syndromes including neurofibromatosis type 1, von Hippel-Lindau disease, multiple endocrine neoplasia type 2 and the succinate dehydrogenase (SDH) syndrome [1].

The SDH complex is a key respiratory enzyme that participates in the Krebs cycle and the electron transport chain and has a role in tumor suppression [3, 4]. The SDH complex comprises the subunits, SDHA, SDHB, SDHC, and SDHD [3, 4]. SDH assembly factor 2 (SDHAF2) encodes a protein that is necessary for flavination and the functioning of SDHA [4]. The SDH complex is assembled at the inner mitochondrial membrane [3, 4]. If any component of this complex (SDHA, SDHB, SDHC, SDHD, and SDHAF2) is inactivated, the entire SDH complex either becomes unstable or does not form and the SDHB subunit is released into the cytoplasm, where it degrades rapidly [5].

Hereditary PCC and PGL are frequently associated with various the SDH gene mutations [6, 7]. Patients with SDHB gene mutations are reportedly at increased risk for malignant PGL [8, 9]. The loss of SDHB expression associated with the SDH gene mutations can be detected rapidly and reliably with immunohistochemistry studies on routinely processed formalin-fixed paraffin-embedded tumor blocks [10, 11].

We previously reported two novel mutations of SDHB, L157X [12] and P236 [13] in PGL. In this study, we performed immunohistochemistry studies for SDHB in two cases of PGL with the SDHB germline mutations, L157X and P236S. In both cases, the results of immunohistochemistry were positive for SDHB. We suggest that immunohistochemistry studies of SDHB might not detect patients with PCC and PGL associated with the SDH complex germline mutations.

SUBJECTS AND METHODS

Tumor samples

In this study, we studied a PGL with SDHB mutation of L157X [12] and a PGL with SDHB mutation of P236S [13]. In addition, we examined five sporadic cases of PCC of the medulla of the adrenal gland. Sporadic PCC was defined as unilateral PCC that did

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not have recurrence of PCC within at least the previous 10 years, with no history of any other tumors and no family history of PCC.

Immunohistochemistry studies of SDHB

Immunohistochemistry studies of SDHB were performed as described previously [11]. The formalin-fixed and paraffin-embedded tissue specimens were cut into 3-μm-thick sections, mounted on adhesive-coated slides, deparaffinized, and hydrated within xylene and ethanol. To block endogenous peroxidase, the specimens were soaked in a solution of 0.3% H_2O_2 methanol for 30 minutes. The slides were then placed in citrate buffer (pH: 6.0) and heated in a microwave oven at 100°C for 15 minutes to unmask the antigen. After cooling, the sections were preincubated in phosphate-buffered saline containing 2% (v/v) normal swine serum (Dako Denmark, Grostrup Hovedstaden, Denmark) for 10 minutes. Immunohistochemistry studies for SDHB were performed with a commercially available mouse monoclonal antibody clone 21A11, isotype immunoglobulin G2a (Abcam, Cambridge, United Kingdom) [10, 11]. The primary antibody was used at a dilution of 1:1000. The slides were then incubated at 4°C overnight with the primary antibody and then washed with phosphate-buffered saline. Dako EnVision horseradish peroxidase (Dako Denmark) was applied for 30 minutes. Diaminobenzidine tetrahydrochloride chromogen was served as the substrate. The sections were counterstained with Harris hematoxylin, rinsed, dehydrated, and covered with coverslips.

Staining patterns were categorized as positive, weak diffuse staining, and negative [11]. Cases that demonstrated definite granular cytoplasmic staining of tumor cells (a mitochondrial pattern) were scored positive. Cases with a very mild cytoplasmic or nuclear blush without the presence of definite granular mitochondrial staining were classified as weak diffuse staining. Cases where the tumor cells were negative were considered informative only when there was a typical mitochondrial pattern of staining in the internal controls (endothelial cells and stromal cells) throughout the slide. Normal adrenal cortex tissue adjacent to the sporadic PCC was used as the external positive control [10]. To obtain negative controls, the slides were incubated with normal mouse immunoglobulin G1 (Dako Denmark) instead of the primary antibody.

RESULTS

The case of PGL with SDHB mutation of L157X showed definite granular cytoplasmic staining of the tumor cells, a mitochondrial pattern, and positive immunohistochemical staining for SDHB (Fig. 1). The case of PGL with SDHB mutation of P236S showed definite granular cytoplasmic staining of the tumor cells, a mitochondrial pattern, and positive immunohistochemical staining for SDHB (Fig. 2). The five cases of sporadic PCC showed definite granular cytoplasmic staining of the tumor cells, a mitochondrial pattern, and positive immunohistochemical staining for SDHB (Fig. 3). Normal adrenal cortex as the external positive control showed definite granular cytoplasmic staining of the cells, a mitochondrial pattern, and positive immunohistochemical staining for SDHB (Fig. 4). The specimens of negative controls were not stained.

DISCUSSION

As described previously, all subunits of the SDH complex (SDHA, SDHB, SDHC, SDHD, and SDHAF2) are tumor suppressor genes inactivated by mutations [14, 15]. If any component of these subunits is inactivated, the entire SDH complex becomes unstable or does not form and SDHB is released into the cytoplasm, where it degrades rapidly and the enzyme activity of the SDH complex is abolished [5]. When the SDHB had a mutation, the level of the SDHB protein did not exactly parallel the abundance of messenger ribonucleic acid, which suggests that the transcription defect of the mutated SDHB could not account entirely for the loss of SDHB expression [16]. The loss of SDHB expression can be detected rapidly and reliably with immunohistochemistry study on routinely processed formalin-fixed, paraffin-embedded blocks of tumor tissue [10, 11]. However, as mentioned, the two PGLs with SDHB mutation of L157X and P236S showed definite granular cytoplasmic staining of SDHB and immunohistochemistry results were positive for SDHB, as previously described [11].

According to two reports, immunohistochemistry studies of 34 cases [10] and 6 cases [11] of PCC and PGL with SDHB mutations yielded negative results for SDHB, without revealing the mutation types of SDHB [10, 11]. Benn *et al.* reported 31 different SDHB mutations spanning exons 2 to 7 of the SDHB gene, including missense, nonsense, frameshift, and splice site mutations, without immunohistochemistry analysis of SDHB [17]. Lima *et al.* showed by crystallography the severe structural consequences of five clinically validated SDHB missense mutations on the SDHB protein, but they too did not perform immunohistochemistry analysis of SDHB [18]. Thus, the relationship among SDHB mutation types, the structural consequences in the SDHB, and the results of immunohistochemistry study of SDHB remained unknown.

PGL associated with SDHB mutation of L157X has been described in two other reports [19, 20]. Immunohistochemistry study for SDHB was not performed in one report [19]; in the other report immunohistochemistry analysis of a head and neck PGL carrying SDHB mutation of L157X revealed positivity for SDHB, with weak diffuse staining [20]. Thus, it was possible that the SDH complex does not necessarily become unstable or release the SDHB into the cytoplasm to degrade in PGLs associated with L157X and P236S mutations in the SDHB.

In recent years, germline mutations of the SDH complex have rarely been associated with solid tumors other than PCC and PGL, such as gastrointestinal stromal tumor [21], renal cell carcinoma [22], and pituitary adenoma [23, 24]. In fact, renal cell carcinoma with germline mutations of the SDH complex is now recognized as a unique and distinct type of renal cell carcinoma in the World Health Organization's 2016 classification [25]. The term "SDH-deficient neoplasia" can therefore be applied to all tumors in which SDHB is not expressed and SDH deficiency can be considered prima facie evidence of syndromic disease, usually germline mutation [26]. Thus, immunohistochemistry study of surrogate markers of SDHB has proved to be strong and reliable for detecting mutations of the SDH

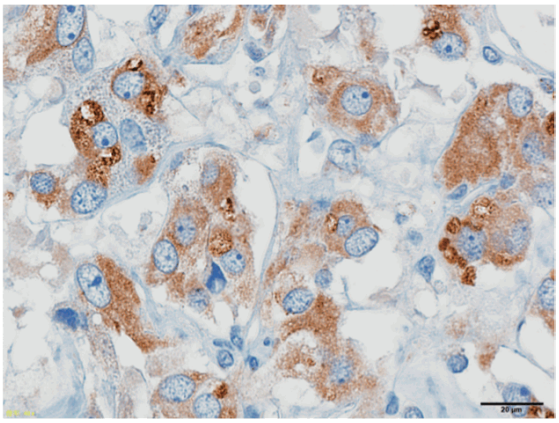

Fig. 1 Paraganglioma with the L157X mutation of succinate dehydrogenase subunit B (SDHB), showing definite granular cytoplasmic staining of the tumor cells and a mitochondrial pattern, as well as immunohistochemical positivity for SDHB.

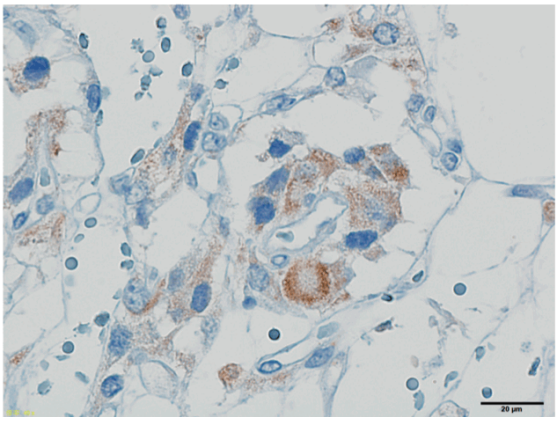

Fig. 2 Paraganglioma with the P236S mutation of succinate dehydrogenase subunit B (SDHB), showing definite granular cytoplasmic staining of the tumor cells and a mitochondrial pattern, as well as immunohistochemical positivity for SDHB.

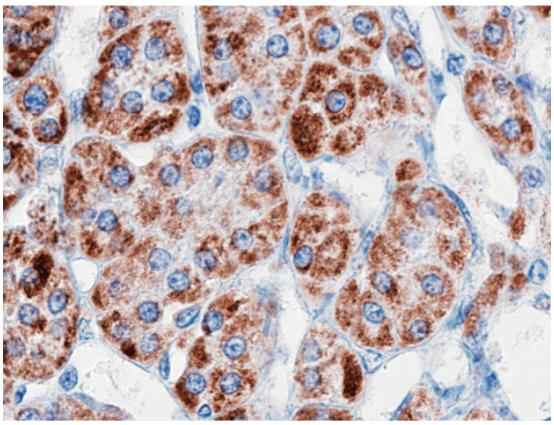

Fig. 3 Sporadic pheochromocytoma showing definite granular cytoplasmic staining of the tumor cells and a mitochondrial pattern, as well as immunohistochemical positivity for succinate dehydrogenase subunit B (SDHB).

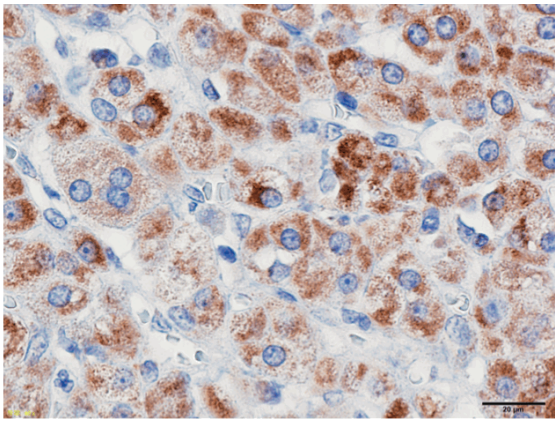

Fig. 4 Normal adrenal cortex tissue (the external positive control) showing definite granular cytoplasmic staining of the cells and a mitochondrial pattern, as well as immunohistochemical positivity for succinate dehydrogenase subunit B (SDHB).

complex in many tumors. However, SDHB mutations of L157X and P236S were not included among the surrogate markers to detect mutations of the SDH complex.

In conclusion, PGLs with SDHB mutations of L157X and P236S showed positive immunohistochemical staining for SDHB. Therefore, the results of immunohistochemistry studies of SDHB should be carefully interpreted for the detection of mutations in SDHA, SDHB, SDHC, SDHD and SDHAF2.

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AUTHOR CONTRIBUTIONS

Haruhiro Sato is a consultant endocrinologist involved in the management of patients. Chie Inomoto is a pathologist who is responsible for pathological diagnosis. Both authors contributed equally to this study and the drafting of the report. Haruhiro Sato has moved from Tokai University School of Medicine to Kanagawa Dental University.

ETHICAL APPROVAL

This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Review Board Committee at Tokai University School of Medicine.

REFERENCES

1) Tischler AS. Pheochromocytoma and extra-paraganglioma updates. Arch Pathol Lab Med 2008; 132: 1272–1284.
2) McNicol AM, Young Jr WF, Kawashima A, Komminoth P, Tischler AS. Benign phaeochromocytoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C eds. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Endocrine Organs. Lyon: IARC Press, 2004: 151–155.
3) Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. Nat Rev Cancer 2011; 11: 325–337.
4) Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. Nat Rev Cancer 2005; 5: 857–866.
5) Ugalde C, Janssen RJ, van de Heuvel LP, Smeitink JA, Nijtmans LG. Differences in assembly or stability of complex I and other mitochondrial OXPHOS complexes in inherited complex I defi-

ciency. Hum Mol Genet 2004; 13: 659–667.
6) Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, *et al*. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 2001; 69: 49–54.
7) Schiavi F, Boedeker CC, Bausch B, Peçzkowska M, Gomez CF, Strassburg T, *et al*. European-American Paraganglioma Study Group. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. JAMA 2005; 294: 2057–2063.
8) Amar L, Bertherat J, Baudin E, Ajzenberg C, Bressac-de Paillerets B, Chabre O, *et al*. Genetic testing in pheochromocytoma or functional paraganglioma. J Clin Oncol 2005; 23: 8812–8818.
9) Brouwers FM, Eisenhofer G, Tao JJ, Kant JA, Adams KT, Linehan WN, *et al*. High frequency of SDHB germline mutations in patients with malignant catecholamine-producing paragangliomas: implications for genetic testing. J Clin Endocrinol Metab 2006; 91: 4505–4509.
10) van Nederveen FH, Gaal J, Favier J, Korpershoek E, Oldenburg RA, de Bruyn EM, *et al*. An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis. Lancet Oncol 2009; 10: 764–771.
11) Gill AJ, Benn DE, Chou A, Clarkson A, Muljono A, Meyer-Rochow GY, *et al*. Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. Hum Pathol 2010; 41: 805–814.
12) Sato H, Kanai G, Hirabayshi K, Kajiwara H, Itoh J, Osamura RY. L157X nonsense mutation of the succinate dehydrogenase subunit B gene in a Japanese patient with right paraaortic paraganglioma. Endocrine. 2010; 38: 18–23.
13) Sato H, Shoji S, Kajiwara H, Itoh J, Osamura RY. A novel mutation (P236S) in the succinate dehydrogenase subunit B gene in a Japanese patient with a posterior mediastinal paraganglioma. Endocr Pathol. 2013; 24: 144–148.
14) Gimenez-Roqueplo AP, Favier J, Rustin P, Rieubland C, Kerlan V, Plouin PF, *et al*. Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. J Clin Endocrinol Metab 2002; 87: 4771–4774.
15) Bardella C, Pollard PJ, Tomlinson I. SDH mutations in cancer. Biochim Biophys Acta 2011; 1807: 1432–1443.
16) Dahia PLM, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, *et al*. A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. PLoS Genet 2005; 1: 72–80.
17) Benn DE, Gimenez-Roqueplo AP, Reilly JR, Bertherat J, Burgess J, Byth K, *et al*. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. J Clin Endocrinol Metab 2006; 91: 827–836.
18) Lima J, Feijão T, da Silva AF, Pereira-Castro I, Fernandez-Ballester G, Máximo V, *et al*. High frequency of germline succinate dehydrogenase mutations in sporadic cervical paragangliomas in northern Spain: mitochondrial succinate dehydrogenase structure-function relationships and clinical-pathological correlations. J Clin Endocrinol Metab 2007; 92: 4853–4864.
19) Saito T, Saito Y, Matsumura K, Tsubota Y, Maniwa T, Kaneda H, *et al*. Novel mutation (L157X) in the succinate dehydrogenase B gene (SDHB) in a Japanese family with abdominal paraganglioma following lung metastasis. Endocr J 2009; 56: 451–458.
20) Takeshima K, Ariyasu H, Uraki S, Kitahara C, Morita S, Inaba H, *et al*. Head and neck paraganglioma atypically carrying a succinate dehydrogenase subunit B mutation (L157X). Intern Med 2020; 59: 1167–1171.
21) Gill AJ, Chou A, Vilain R, Clarkson A, Lui M, Jin R, *et al*. Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. Am J Surg Pathol 2010; 34: 636–644.
22) Gill AJ, Hes O, Papathomas T, Šedivcová M, Tan PH, Agaimy A, *et al*. Succinate dehydrogenase (SDH) deficient renal carcinoma – a morphologically distinct entity: a clinicopathologic series of 36 tumors from 27 patients. Am J Surg Pathol 2014; 38: 1588–1602.
23) Xekouki P, Stratakis CA. Succinate dehydrogenase (SDHx) mutations in pituitary tumors: Could this be a new role for mitochondrial complex II and/or Krebs cycle defects? Endocr Relat Cancer 2012; 19: C33–40.
24) Xekouki P, Pacak K, Almeida M, Wassif CA, Rustin P, Nesterova M, *et al*. Succinate dehydrogenase (SDH) D subunit (SDHD) inactivation in a growth-hormone-producing pituitary tumor: A new association for SDH? J Clin Endocrinol Metab 2012; 97: E357–366.
25) Gill AJ, Amin MB, Smith SC, Trpkov K. Succinate dehydrogenase (SDH) deficient renal carcinoma. In: Moch H, Humphrey PA, Ulbright TM, Reuter VE eds. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press, 2016: 35–36.
26) Gill AJ. Succinate dehydrogenase (SDH)-deficit neoplasia. Histopathol 2018; 72: 106–116.