Novel Mutation (L157X) in the Succinate Dehydrogenase B Gene (SDHB) in a Japanese Family with Abdominal Paraganglioma Following Lung Metastasis

TOMOHITO SAITO*, YUKIHITO SAITO*, KOICHIRO MATSUMURA*, YU TSUBOTA*, TOMOHIRO MANIWA*, HIROYUKI KANEDA*, KEN-ICHIRO MINAMI*, NORIKO SAKAIDA**, YOSHIKO UEMURA**, GEN KAWA***, NAE YAMAMOTO***, YOSHIMITSU FUJII†, KAZUMASA ISOBE‡, YASUSHI KAWAKAMI‡, TADASHI MATSUDA*** and KAZUHIRO TAKEKOSHI∗

* Division of Thoracic Surgery, Department of Thoracic and Cardiovascular Surgery, Kansai Medical University, Osaka, Japan
** Department of Surgical Pathology, Kansai Medical University, Osaka, Japan
*** Department of Urology, Kansai Medical University, Osaka, Japan
† Nakano Kodomo Hospital, Osaka, Japan
‡ Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan

Abstract. Recently, nuclear genes encoding two mitochondrial complex II subunit proteins, SDHD and SDHB, have been found to be associated with the development of familial pheochromocytomas and paragangliomas (hereditary pheochromocytoma/paraganglioma syndrome: HPPS) [1, 2]. Growing evidence suggests that a mutation in SDHB is highly associated with abdominal paraganglioma and the subsequent distant metastasis (malignant paraganglioma). In the present study, we report the case of a novel SDHB mutation (L157X) in a Japanese patient with abdominal paraganglioma following malignant lung metastasis. In addition, we identified an asymptomatic carrier of the SDHB mutation in this family.

Key words: paraganglioma, SDHB

THE NUCLEAR genes encoding two mitochondrial complex II subunit proteins, SDHD and SDHB, have been reported to be associated with the development of familial pheochromocytomas and paragangliomas (hereditary pheochromocytoma/paraganglioma syndrome: HPPS) [1, 2]. Growing evidence suggests that a mutation in SDHB is highly associated with abdominal paraganglioma and the subsequent distant metastasis (malignant paraganglioma) [3-8]. Indeed, it has been found that malignant pheochromocytoma/paraganglioma is associated with 38 to 83% of patients with SDHB germline mutations, indicating a much higher rate of malignancy compared with other mutations or sporadic adrenal pheochromocytoma where the rate is <10%. In contrast, among all patients with malignant pheochromocytoma/paraganglioma the frequency of SDHB mutations is reported to be around one third, suggesting that SDHB mutations in these malignant tumors may not be as rare as expected [9].

Previously, we identified a novel heterozygous G to A point mutation at the first base of intron 3 of the SDHB gene (IVS3+1G>A) in addition to the known R46Q mutation in malignant abdominal paraganglioma from two Japanese families, respectively [10, 11].

Here we report the case of a novel SDHB mutation (C.470delT :DB-ID SDHB_00121:L157X) in a Japanese patient with abdominal paraganglioma following lung metastasis. In addition, we identified an asymptomatic carrier of the SDHB mutation in this family.
Subjects and methods

Patient and family members

A 56 year-old Japanese male presented to our institute with a two-year history of weight loss and abdominal discomfort. No familial incidence of pheochromocytoma or paraganglioma was known in the kindred (Fig. 1). Hypertension and hyperglycemia emerged one year before admission. On admission, the patient’s urinary norepinephrine level was extremely elevated (4583.6 μg per 24 h). Urinary dopamine and normetanephrine levels were also elevated as shown in Table 1. Enhanced abdominal computed tomography (CT) revealed a paraaortic tumor, which showed high signal intensity on T2-weighted MR images (Fig. 2-A,B). There was an abnormal uptake at the tumor site in MIBG scintigraphy. The tumor was completely resected, and extraadrenal paraganglioma was diagnosed through pathological investigation (Fig. 3). Abdominal discomfort, hypertension, and hyperglycemia diminished postoperatively. Two years later, the patient also received a pancreatoduodenectomy for duodenal papillary adenocarcinoma.

Seven years after the first operation for retroperitoneal paraganglioma, multiple nodules in the bilateral lungs were detected by chest CT without any symptoms (Fig. 4-A). Fluorodeoxyglucose positron emission tomography (FDG-PET) showed high uptake at the nodules in S8 of both lungs (Fig. 4-B). We resected two nodules in the right lower lobe of the lung, both of which were diagnosed as pulmonary metastasis of paraganglioma (Fig. 5)[12]. Therefore, malignant extraadrenal paraganglioma was also diagnosed in this case. We also performed metastasectomy for the nodules in the left lower lobe of the lung six months later. Postoperative hypotension was observed, but the patient was discharged without any other complications, indicating that metastasectomy was carried out successfully. However, in case further metastasis emerge, MIBG radiotherapy and/or cytotoxic chemotherapy with cyclophosphamide, vincristine and dacarbazine should be considered. His clinical manifestations raised concerns about the presence of a germline mutation in the SDHB gene.

After informed consent, we carried out genetic analysis of the proband as well as his two daughters (details are given in Genetic analysis described below). Consequently, the SDHB mutation (L157X)
Fig. 3. Pathological findings of the resected retroperitoneal tumor.

A. Gross appearance of the tumor. The size was 55 x 51 x 43 mm, and the weight was 60 g.
B. Gross section of the tumor. Macroscopically, sclerotic change was observed in the central area, which has a fine border with the surrounding tissue.
C. Microscopic findings showed a trabecular arrangement of tumor cells with small round nuclei and basophilic cytoplasm. Nuclear pleomorphism and apoptosis were also found. The tumor cells had argyrophilic features and were positive for chromogranin A.
D. Vascular invasion of tumor cells was identified. Microinvasion to either fibrous capsule or necrotic foci was also observed in this tumor.

Fig. 4. Computed tomography of the thorax seven years after the first operation.
Chest CT shows four pulmonary nodules. The nodules are located in S6 and S8 of the bilateral lungs. FDG-PET shows high uptake at the nodule sites in S8 of the bilateral lungs.
Peripheral blood for germline DNA analysis was drawn from the family members after written informed consent was obtained. Using blood DNA, the eight exons of the SDHB gene were screened with intronic primers [10]. PCR was carried out as described previously [10, 11].

PCR amplicons were column purified and subjected to semi-automated sequencing using the above

**Genetic analysis**

Participants in this study were informed about the possibility of a genetic study, its implications, and its purpose. A written informed consent was obtained from those wishing to participate in the study, and the study was approved by the ethics committee of the Medical Faculty of Tsukuba University, Tsukuba, Japan. Blood samples were collected from the participants and DNA extracted using a Blood DNA extraction kit (WAKO, Osaka, Japan).

**PCR and Sequence analysis**

Peripheral blood for germline DNA analysis was drawn from the family members after written informed consent was obtained. Using blood DNA, the eight exons of the SDHB gene were screened with intronic primers [10]. PCR was carried out as described previously [10, 11].

PCR amplicons were column purified and subjected to semi-automated sequencing using the above
Discussion

In this study, we identified a novel germline mutation in the \textit{SDHB} gene (c.470delT : L157X) in a malignant paraganglioma patient, as well as in his second daughter, who seemed to be asymptomatic. It would appear that this novel mutation is a disease-causing mutation since the heterozygous T nucleotide deletion resulted in a stop codon and a predicted truncated protein (L157X).

Current evidence suggests that mutations in \textit{SDHB} are frequently related to abdominal paraganglioma and the following distant metastasis, which is in agreement with the case presented here [3-8]. Furthermore, the identification of \textit{SDHB} mutations reported to be predictive of rapid metastatic spread, in cases in which malignancy is documented, indicating that identification of \textit{SDHB} mutations might allow for more patient-tailored management [13]. Therefore, it is rec-
ommended that all patients with metastatic disease, especially from paraganglioma, be tested for SDHB mutations.

Although penetrance of SDHB is reported to be high (approximately 90%), SDHB mutations are often detected in patients with apparently sporadic pheochromocytoma (ASP), again consistent with our case [3, 6, 7]. Indeed, only 10% to 30% of familial paraganglioma have a family history [14]. Consistent with these findings, Benn et al. reported SDHB mutations in 3 families in which penetrance was variable [15]. In these three families, the SDHB mutation-positive parent of each proband had no disease symptoms, yet the proband manifested the disease at an early age (12, 15 and 7 years). This pattern of disease presentation suggests that SDHB may be a variably penetrant gene with its expression being influenced by genetic or environmental modifiers. At present, however, the genetic or environmental factors responsible for this large variability in penetrance remain to be identified [15]. Thus, we agree that a negative family history of pheochromocytoma and paraganglioma by no means rules out the presence of a SDHB mutation.

Recently, a detailed description of the clinical presentations, biochemical phenotypes and genotype-phenotype correlations of patients with mutations in SDHB was reported by Timmers et al. [14].

In more than half of the patients analyzed by these authors, the clinical picture at the initial presentation was dominated by problems caused by the space-occupying effect of the tumor, including pain and discomfort, and weight loss [14]. The biochemical phenotype was hypersecretion of both norepinephrine and dopamine in approximately half of the cases [14].

In agreement with their reports [14], the clinical picture at the initial presentation of our patient was abdominal discomfort. Also, the biochemical phenotype was consistent with hypersecretion of both norepinephrine and dopamine (Table 1). Thus, these clinical manifestations and biochemical phenotypes should make clinicians to think about the possibility of SDHB-related tumors.

Malignant tumors are typically large, have a higher mitotic count, and have extensive local or vascular invasion. For our patient, the pathological findings in the primary tumor identified vascular invasion of tumor cells (Fig. 3-D), which is highly suggestive of malignant disease. However, our case may be rather unusual, since tumor invasion of blood/lymphatic vessels or adjacent organs was mentioned in the pathology report of primary tumors in 14 (48%) of 29 SDHB-related malignant cases. Thus, in SDHB-related malignant cases, it can also be suggested that pathological examination appears to be of limited value for predicting metastasis.

Recently, Kimura et al. demonstrated that their pathology scoring system, which identified the tumor differentiation, significantly correlates with both metastatic potential and patient survival [16]. Considering that SDHB-mutations associate with malignant potential, it raises the possibility that incorporation of this genetic testing with a pathology scoring system might involve more definitive assessment of malignancy, genotype-phenotype correlation and identification of target for therapy [16].

Recently, it is proposed that presymptomatic genetic testing should be offered to all first-degree relatives of an individual in whom a mutation in SDHB has been detected [6, 7, 17]. Therefore, another important finding of this study was to identify the same SDHB mutation (L157X) in the second daughter of the proband as an asymptomatic carrier. At present, asymptomatic carriers of SDHB have been demonstrated in two large studies in the literature [6, 7]. Although there is little information available on how to follow-up and/or treat asymptomatic carriers, recommendations have been proposed as a result of these two studies, as described above [6, 7, 17]. The other critical

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Result</th>
<th>Reference range</th>
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<tr>
<td>Urinary catecholamines</td>
<td></td>
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<tr>
<td>Epinephrine (µg/24hr)</td>
<td>6.1</td>
<td>3.4-26.9</td>
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<tr>
<td>Norepinephrine (µg/24hr)</td>
<td>458.3</td>
<td>48.6-168.4</td>
</tr>
<tr>
<td>Dopamine (µg/24hr)</td>
<td>982.5</td>
<td>365-961.5</td>
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<tr>
<td>Urinary deconjugated fractionated metanephrines</td>
<td></td>
<td></td>
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<tr>
<td>Metanephrine (mg/24hr)</td>
<td>0.11</td>
<td>0.04-0.19</td>
</tr>
<tr>
<td>Normetanephrine (mg/24hr)</td>
<td>5.44</td>
<td>0.09-0.33</td>
</tr>
<tr>
<td>Vanillyl mandelic acid (mg/24hr)</td>
<td>25.6</td>
<td>1.5-4.3</td>
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Table 1. Biochemical findings on the first admission.

Urinary norepinephrine and normetanephrine were extremely elevated. Urinary dopamine was slightly elevated. The amounts of urinary catecholamines, urinary deconjugated fractionated metanephrines, and vanillyl mandelic acid were measured by high-performance liquid chromatography (HPLC).
point of the recommended protocol is that all subjects must undergo periodic surveillance regardless of the presence of signs and symptoms, since it may lead to early detection and tumor removal. However, it remains unclear whether early detection and tumor removal may prevent any subsequent development of a fatal malignancy. Prior to presymptomatic genetic testing, a written informed consent including these limitations of this testing was obtained from the two daughters of the proband as individuals at risk. Also from the viewpoint of genetic counseling, it is highly important to correctly inform these at-risk individuals as to these limitations and help them make informed medical and personal decisions.

In the US, most SDHB mutation carriers are subject to follow-up by National Institutes of Health (NIH), where there are many clinicians experienced with pheochromocytoma and paraganglioma. Thus, we believe that in Japan SDHB mutation carriers, especially asymptomatic individuals, should be followed-up over an extended period of time at a restricted number of facilities where clinicians who specialize in pheochromocytoma and paraganglioma are working.

Further studies, especially prospective follow-up analyses of the SDHB mutation, are needed to establish routine screening for pheochromocytoma/paraganglioma, including asymptomatic carriers.

References


