Clinicopathologic Characteristics and Mutational Status of Succinate Dehydrogenase Genes in Paraganglioma of the Urinary Bladder

A Multi-Institutional Korean Study

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• ***Context.***—**Because of the limited number of available primary bladder paraganglioma (PBPG) cases, the rates of succinate dehydrogenase (*SDH*) mutations and the clinicopathologic characteristics of SDH-deficient tumors have not been fully studied.**

Objective.—**To define the clinicopathologic and molecular characteristics of PBPGs.**

Design.—**A total of 52 PBPGs were collected retrospectively. SDHA and SDHB immunohistochemical stains were performed. In cases of SDHB expression loss, mutation analyses of *SDHB*, *SDHC*, and *SDHD* were performed.**

Results.—**The clinicopathologic features were analyzed for 52 cases (M:F = 27:25), with a mean age of 56 years (range, 22–79 years). Tumor sizes were 0.5 to 8 cm (mean, 2.4 cm). Tumor necrosis was present in 5 of 52 cases (10%), involvement of muscularis propria in 41 (79%), and lymphovascular tumor invasion in 6 (12%). During a mean follow-up period of 41 months (range, 1–161 months), 3 of 52 patients (6%) developed metastases, but no one died from the disease. Immunohistochemistry for SDHA and SDHB showed that all cases were SDHA intact. Among them, 43 cases had intact SDHB, whereas 9 cases were SDHB deficient. Compared with the SDHB-intact cases, the SDHB-deficient cases were characterized by large tumor sizes (4.5 versus 1.9 cm; $P < .001$), a higher number of mitoses per 10 high-powered fields (2.6 versus 0.1; $P = .002$), and frequent lymphovascular tumor invasion (33% versus 7%; $P = .02$) and metastases (22% versus 2%; $P = .02$). Mutational analyses for *SDHB*, *SDHC*, and *SDHD* were performed in 9 SDHB-deficient cases. Among them, 6 cases were successfully sequenced and revealed *SDHB* mutations only.**

Conclusions.—**Large tumor size, a higher number of mitoses, and the presence of lymphovascular tumor invasion and *SDHB* mutations suggest malignant paraganglioma.**

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Paragangliomas (PGs) consist of sympathetic and parasympathetic neoplasms, depending on tumor location and catecholamine production. Parasympathetic PGs are located in the head and neck region, and they usually do not

produce catecholamines, whereas sympathetic PGs are located in the sympathetic trunk of the abdomen and usually produce catecholamines.[1] Most PGs are located in the head and neck (69%), followed by the abdominal (21.5%) and thoracic (9.5%) areas.[2] Paragangliomas can arise sporadically and in the context of several inherited tumor syndromes.[3] Studies have demonstrated that pheochromocytoma-paraganglioma syndrome is a hereditary condition frequently associated with PGs caused by germ line mutations in the *SDHB*, *SDHC*, or *SDHD* genes, encoding 3 of the 4 subunits of mitochondrial complex II, the succinate-ubiquinone oxidoreductase (succinate dehydrogenase [*SDH*]). The enzyme *SDH* is located at the crossroads between the aerobic electron transport chain and the tricarboxylic acid cycle.[4] In contrast to head and neck PGs, where the *SDHD* mutation is the most frequent, followed by *SDHB* and *SDHC*,[5,6] the *SDHB* mutation is more dominant in thoracoabdominal PGs.[6,7] Several previous reports have indicated that the recurrence rate and degree of malignancy of PGs are higher in cases of *SDHB* mutation.[7–10] Therefore, detecting *SDHB* mutations in thoracoabdominal PGs is crucial for prognosis, because there are no reliable histologic characteristics to predict malignancy reported to date.

It was demonstrated that an absence of SDHB staining in tumor cells indicates underlying *SDH* germ line mutations with high reliability.[11] In contrast, a loss of SDHA staining in tumor cells has been observed only in *SDHA* mutations.[12] Concurrent loss of SDHA and SDHB staining is encountered in tumors with only *SDHA* mutations, but a loss of only SDHB staining is found in tumors with all *SDH* mutations, except for the *SDHA* mutation. Therefore, it is recommended that *SDHA* sequencing be performed if a tumor shows a loss of both SDHA and SDHB stains. Sequencing of *SDHB*, *SDHC*, or *SDHD* is required if a tumor shows a loss of SDHB staining and intact SDHA staining.[13]

Primary bladder PGs (PBPGs) are extremely rare, accounting for less than 0.05% of all bladder tumors.[14] Because of the limited number of available PBPG cases, the rates of *SDH* mutations and clinicopathologic characteristics of SDH-deficient tumors have not been fully determined. Herein, we report a retrospective study that was performed using 52 PBPG cases collected from major institutions in Korea.

MATERIALS AND METHODS

Pathology and Medical Record Review

A search of pathology databases was performed for PBPGs from 19 institutions—namely, Ewha Womans University Mok-dong Hospital (Seoul), Samsung Medical Center (Seoul), Ajou University Hospital (Suwon), Yonsei University Severance Hospital (Seoul), Korea University Anam Hospital (Seoul), Inje University Haeundae Paik Hospital (Pusan), Inje University Sanggye Paik Hospital (Seoul), Seoul National University Hospital (Seoul), Hanyang University Guri Hospital (Guri), Pusan National University Hospital (Pusan), Incheon St Mary's Hospital (Incheon), Kyung Hee University Medical Center (Seoul), Dankook University Hospital (Cheonan), Dongguk University Hospital (Gyeonju), Inje University Busan Paik Hospital (Pusan), Asan Medical Center (Seoul), Gachon University Gil Medical Center (Incheon), Konyang University Hospital (Daejeon), Seoul National University Bundang Hospital (Sungnam), and Seoul St Mary's Hospital (Seoul)—in South Korea. After reviewing all available hematoxylin-eosin–stained slides, either one representative paraffin block from each case or additional unstained slides from each case were collected from each institution for further immunohistochemical and *SDH* mutational studies. Clinical data for each case included age at diagnosis, sex, personal tumor history, family tumor history, and clinical outcome. Pathology data for each case included type of specimen, tumor location, mitotic count, presence of necrosis, lymphovascular invasion and proper muscle invasion, and immunohistochemical staining results (when available). The Institutional Review Board at Ewha Womans University Hospital approved the study.

Immunohistochemical Analysis

All immunohistochemical stains for SDHA and SDHB were performed on 4-μm–thick, formalin-fixed, paraffin-embedded whole-tissue sections provided from 19 institutions after pressure cooker antigen retrieval (0.001 M citrate buffer; pH 6.0), using mouse anti-SDHA (1:700 dilution; 40-minute incubation; clone 2E3GC12FB2AE2, Abcam, Cambridge, Massachusetts) and anti-SDHB (1:100 dilution; 40-minute incubation; clone 21A11AE7, Abcam) monoclonal antibodies, as previously described.[13] One case of pheochromocytoma with a known *SDHB* mutation, and one with a known *SDHD* mutation, were used as controls for tumors with loss of SDHB immunohistochemical staining.[15,16] For a negative control used for SDHA antibody, dilution buffer was used instead of the primary antibody. Expression was evaluated as "intact" when granular cytoplasmic staining was observed in tumor cells. On the other hand, expression was evaluated as "deficient" when there was a complete absence of granular cytoplasmic staining in tumor cells along with positive staining in the internal positive control cells, such as sustentacular or proper muscle cells of the urinary bladder.

Sequence Analysis

In cases of SDHB expression loss, mutation analyses of *SDHB*, *SDHC*, and *SDHD* were performed. Genomic DNA was isolated from the tumor using a QIAamp DNA FFPE tissue kit (Qiagen, Valencia, California). Tumor DNA was amplified using primers for exons 8, 6, and 4 of *SDHB*, *SDHC*, and *SDHD*, respectively (primers used are listed in Table 1). Normal corresponding tissues were not available. Bidirectional sequencing was performed using the BigDye Terminator v1.1 kit (Applied Biosystems, Foster City, California) on an ABI 3130xl genetic analyzer (Applied Biosystems). Sequencer version 4.10.1 (Gene Codes Corporation, Ann Arbor, Michigan) was used, along with manual chromatogram reviews for sequence analysis. Confirmatory resequencing from replicate polymerase chain reaction (PCR) was performed for sequences that were ambiguous or deviated from wild-type, so that all abnormal sequences were confirmed in at least quadruplicate for replicate amplification reactions. The results were marked as mutation-positive if a mutation was detected in both the forward and reverse DNA strands.

RESULTS

Clinicopathologic Characteristics

Clinicopathologic data are summarized in Table 2. The patient population (N = 52) included 27 male (52%) and 25 female (48%) patients, with a mean age of 56 years at diagnosis (range, 22–79 years). At the time of diagnosis, patients with SDHB-deficient tumors were relatively younger than patients with SDHB-intact tumors (means, 43 and 59 years, respectively; $P = .002$). All patients presented with a single isolated tumor. Tumor sizes were available in 50 of the 52 cases, including 41 SDHB-intact and 9 SDHB-deficient cases. The mean tumor size was 2.4 cm, and it was larger for SDHB-deficient tumors compared with SDHB-intact tumors (means, 4.5 and 1.9 cm, respectively; $P <$

Table 1. Primer Sequences and Polymerase Chain Reaction Conditions for Detection of *SDHB*, *SDHC*, and *SDHD*

Primer Names	Forward Primer Sequence	Reverse Primer Sequence	Ta, °C
SDHB EX1	GGTCCTCAGTGGATGTAGGC	CTTGCCCTATGCTTCCTCAG	58
SDHB EX2	TGGATATTGAATGCCTGCCT	GCCTTCCAAGGATGTGAAAA	58
SDHB EX3	ACATCCAGGTGTCTCCGATT	CCCACGTACCTTCTCTGCAT	58
SDHB EX4	TGGATATTGAATGCCTGCCT	ACAAATCCTGCCCTGAAAAA	58
SDHB EX5	CAGTGTCCAAGAAATGGGGT	TGCCAGTTCCTCTCCAGAAT	58
SDHB EX6	GCACTGACCCCAAAGGTAAC	ATGGCAATGAAGGAAACCAG	58
SDHB EX7	CCAGAGCTTTGAGTTGAGCC	TGGTCCCTTTCCTTCTCAAA	58
SDHB EX8	AACCCCTATGGTTTTGAGGG	TGCTGTATTCATGGAAAACCAA	58
SDHC EX1	GTCACATGACACCCCCAAC	CCCAGGCACAGGATAAACAG	60
SDHC EX2	TCTATCCCTTCACCCCTAAAAA	CGCCTGTAGTCCCAGCTACT	60
SDHC EX3	TTTTCAAACGGTCTGGTTTT	TGGTTGAGTAAAAGTGAGGGAAG	62
SDHC EX4	GAGCTGAGATCATGCCATTG	TTCAAAGGAGGCGGAGACTA	62
SDHC EX5	CAGGGGTCCCAGTTTTATGT	AGTCTCCCCACTCCCTTCAC	58
SDHC EX6	CGCTTTTCTCTAGAATCATGCTG	TTCCCAGGCTGGAGATAAGA	58
SDHD EX1	TTCACCCAGCATTTCCTCTT	CTGGAGGCTACGCTAAGCAC	58
SDHD EX2	TCAGTCCTGTTAAAGGAGAGGTTC	CCCCCTACAGGTAGGAAGTC	58
SDHD EX3	TTTGGGTTACTGTGTGGCATA	CACAGCAAACAAACTGAGCA	58
SDHD EX4	TTTTTGCAGCCAAGTTATCTGT	CATGACAAAGCAGAGGCAA	58

Abbreviation: Ta, annealing temperature.

.001). A nested, zellballen pattern was present in all cases (Figure 1, A). The mean number of mitoses per 10 high-power fields was 0.3, and it was higher for SDHB-deficient tumors compared with SDHB-intact tumors (means, 2.6 and 0.1, respectively; $P = .002$). One atypical mitosis was identified in a case of an SDHB-deficient tumor (Figure 1, B). Of 52 cases, tumor necrosis was present in 5 cases (10%), proper muscle invasion was present in 41 cases (79%), and

Table 2. Clinicopathologic Characteristics of Paragangliomas of Bladder[a]

Characteristics	Total Cohort (N = 52)	SDHB Intact (n = 43)	SDHB Deficient			*P*[b]
			Total (n = 9)	Without Metastases (n = 7)	With Metastases (n = 2)	
Sex, No. (%)						.09[c]
Female	25 (48)	23 (53)	2 (22)	2 (29)	0 (0)	
Male	27 (52)	20 (47)	7 (78)	5 (71)	2 (100)	
Age at diagnosis, y						.002[d]
Mean	56	59	43	45	36.5	
Range	22–79	34–79	22–65	26–65	22–51	
Isolated bladder PGL, No. (%)						
Yes	52 (100)	43 (100)	9 (100)	7 (100)	2 (100)	
No	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Tumor characteristics						
Size, cm, mean	2.4	1.9[c]	4.5	3.7	7.3	<.001[e]
Mitoses, mean per 10 HPF	0.3	0.1	2.6	1.1	4	.002[f]
Necrosis, No. (%)	5 (10)	4 (9)	1 (11)	0 (0)	1 (50)	.87[c]
Lymphovascular invasion, No. (%)	6 (12)	3 (7)	3 (33)	2 (29)	1 (50)	.02[c]
Proper muscle invasion, No. (%)	41 (79)	32 (74)	9 (100)	7 (100)	2 (100)	.09[c]
Metastatic disease, No. (%)						.02[c]
No	49 (94)	42 (98)	7 (78)	7 (100)	0 (0)	
Yes	3 (6)	1 (2)	2 (22)	0 (0)	2 (100)	
Systemic metastases	2 (4)	1 (2)	1 (11)	0 (0)	1 (100)	
Nodal metastases	2 (4)	1 (2)	1 (11)	0 (0)	1 (100)	
Family history of PGL						
No	52 (100)	43 (100)	9 (100)	7 (100)	2 (100)	
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
SDH mutational analysis, No. (%)[g]						
SDHB mutation			6 (100)	4 (100)	2 (100)	
SDHB wild type			0 (0)	0 (0)	0 (0)	

Abbreviations: HPF, high-power field; PGL, paraganglioma.

[a] Tumor size was available for 41 of 43 SDHB-intact cases.
[b] SDHB intact (n = 43) versus SDHB deficient (n = 9).
[c] Chi-square test.
[d] By *t* test.
[e] Mann-Whitney.
[f] Fisher exact test.
[g] SDH mutational analysis was successfully performed in 6 of 9 SDHB-deficient cases.

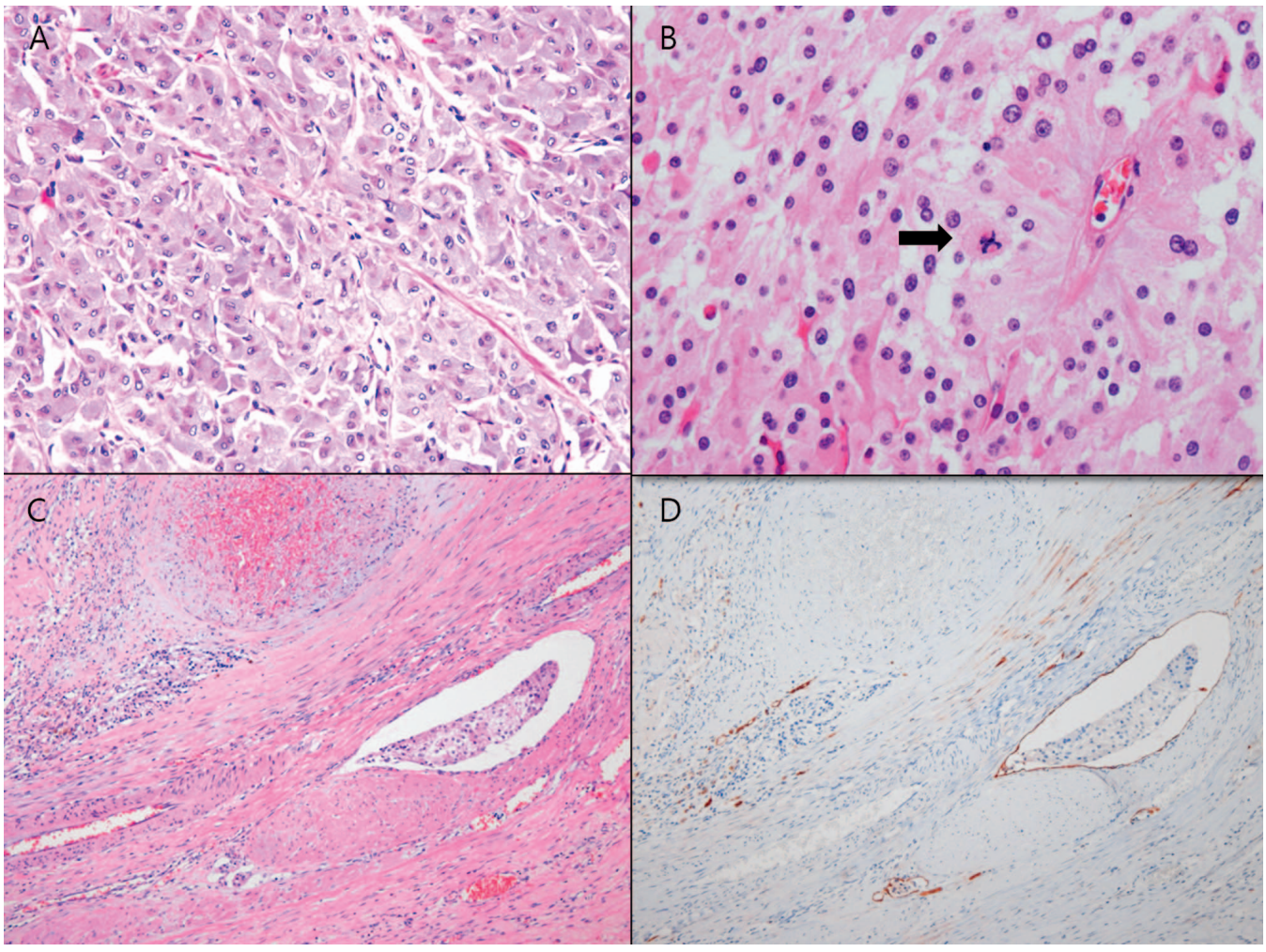

Figure 1. *Examples of SDHB-deficient paragangliomas. A, Hematoxylin-eosin–stained slide. B, Atypical mitosis (arrow). C, Lymphovascular invasion. D, Lymphovascular invasion highlighted by immunohistochemical staining (original magnification ×200 [A]; hematoxylin-eosin, original magnifications ×400 [B] and ×100 [C]; D2-40, original magnification ×100 [D]).*

lymphovascular invasion (LVI) was present in 6 cases (12%; Figure 1, C and D). The presence of LVI was found to occur more frequently in SDHB-deficient cases compared with SDHB-intact tumors (3 of 9; 33%; and 3 of 43; 7%; respectively; $P = .02$). The presence or absence of tumor necrosis and proper muscle invasion, as well as tumor cell morphology (polygonal versus spindle), nuclear size, and nuclear pleomorphism, were not associated with SDHB status or malignant PGs. Only 3 of 52 patients (6%) developed metastases during a follow-up period of 41 months (range, 1–161 months): 1 had intact SDHB and 2 were SDHB deficient. A single SDHB-intact patient had bone and lymph node metastases 3 years after initial diagnosis, an SDHB-deficient patient had lymph node metastases at the time of diagnosis, and a second SDHB-deficient patient had multiple bone metastases 8 years after the initial diagnosis. None had a family history of PGs. Comparison of benign and malignant PGs is shown in Table 3. The mean tumor size was 2.4 cm, and it was larger for malignant PGs compared with benign PGs (means, 6.2 and 2.1 cm, respectively; $P = .002$). Presence of LVI was found to occur more frequently in malignant PGs compared with benign PGs (2 of 3; 67%; and 4 of 49; 8%; respectively; $P = .03$).

Results of Immunohistochemical Analyses

SDHA and SDHB immunostaining was performed in all 52 cases, and all showed intact staining for SDHA. Of 52 cases, 9 cases (17.3%) showed a loss of SDHB expression. The remaining 43 cases (83%) showed intact staining for both SDHA and SDHB. Representative images of SDHA and SDHB staining are shown in Figure 2. Homogeneous cytoplasmic granular staining of SDHA and SDHB was observed throughout the tumors in all positive cases (Figure 2, A). Loss of SDHB staining was observed uniformly in the tumor cells, compared with positive staining of the internal control cells (endothelial, sustentacular, and proper muscle cells; Figure 2, B).

Results of Sequencing Analyses

Genetic analyses detected *SDHB* mutations in all 6 SDHB-deficient tumors that were successfully sequenced. The results of the sequencing analyses for the 6 cases were as follows: in the first case, 4 missense mutations were detected in exons 3 and 8. A heterozygous single–base pair substitution was found at 2 points of exon 3, c.233 A>G

Table 3. Comparison of Benign and Malignant Paragangliomas of Bladder[a]				
Characteristics	Total Cohort (N = 52)	Benign PG (n = 49)	Malignant PG (n = 3)	*P*
Sex, No. (%)				
Female	25 (48)	25 (51)	0 (0)	.24[b]
Male	27 (52)	24 (49)	3 (100)	
Age at diagnosis, y				.11[c]
Mean	56	57	41	
Range	22–79	26–79	22–51	
Isolated bladder PG, No. (%)				
Yes	52 (100)	49 (100)	3 (100)	
No	0 (0)	0 (0)	0 (0)	
Tumor characteristics				
Size, cm, mean	2.4	2.1	6.2	.002[c]
Mitoses, mean per 10 HPF	0.3	0.2	1	.14[c]
Necrosis, No. (%)	5 (10)	4 (8)	1 (33)	.27[b]
Lymphovascular invasion, No. (%)	6 (12)	4 (8)	2 (67)	.03[b]
Proper muscle invasion, No. (%)	41 (79)	38 (78)	3 (100)	>.99[b]
Family history of PG, No. (%)				
No	52 (100)	49 (100)	3 (100)	
Yes	0 (0)	0 (0)	0 (0)	
SDHB IHC, No. (%)				.07[b]
Intact	43 (83)	42 (86)	1 (33)	
Deficient	9 (17.3)	7 (14)	2 (67)	
SDHB mutation, No. (%)				.003[b]
Mutation	6	4	2	
Not done	46	45	1	

Abbreviations: HPF, high-power field; IHC, immunohistochemistry; PG, paraganglioma.

[a] Tumor size was available for 50 cases (47 benign, 3 malignant).
[b] Fisher exact test.
[c] Mann-Whitney.

(p.K78R) and c.241 A>G (p.N81D); and at 2 points of exon 8, c.776 C>T (p.P259L) and c.818 A>G (p.Y273S). In the second case, 2 missense mutations were detected at exon 6, c.571 T>A (p.C191S) and c.578 G>A (p.S193N). In the third case, 1 missense mutation was detected at exon 3, c.221 T>C (p.M71T). In the fourth case, 1 silent mutation was detected at exon 1, c.171C>T (p.T57T). In the fifth case, 1 silent mutation was detected at exon 3, c.225T>C (p.A75A). In the sixth case, 1 intronic mutation was detected at exon 1, c.72+24G>A; and 1 silence mutation at exon 3, c.225T>C (p.A75A). Features of *SDHB*-mutated PBPGs are summarized in Table 4.

DISCUSSION

Primary bladder PGs are rare, with an incidence of only 0.67% (2 of 297) of total PGs reported.[2] Moreover, malignant PBPGs in particular are extremely rare, and the incidence of malignancy is not well known, because the

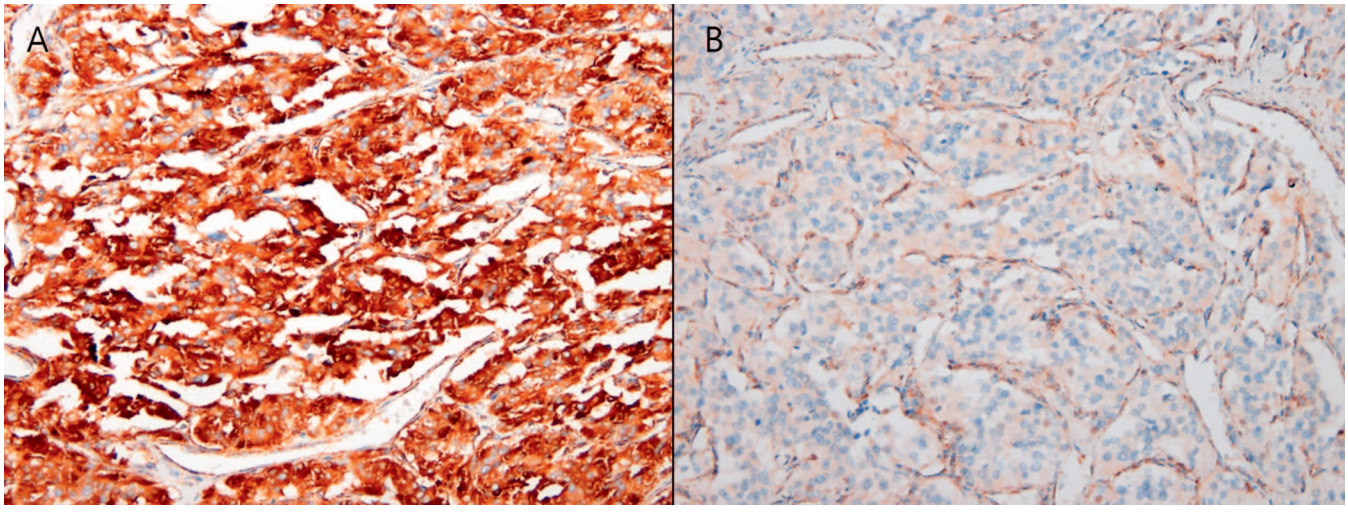

Figure 2. *A, Immunohistochemical staining for SDHA in a paraganglioma with intact expression of SDHB. Staining is cytoplasmic and granular. B, Immunohistochemical staining for SDHB in a paraganglioma with loss of SDHB expression. Sustentacular cells show positive staining as an internal positive control (original magnifications ×100 [A] and ×200 [B]).*

Table 4. Features of 6 *SDHB*-Mutated Paragangliomas of Bladder

Case No.	Age, y/Sex	Size, cm	Atypical Mitosis	Mitotic Count, HPF	LVI	Follow-up, mo	*SDHB* Mutational Status	Metastasis
1	56/M	4.6	−	0	−	21	4 missense mutations: exon 3, c.233 A>G (p.K78R) and c.241 A>G (p.N81D); and exon 8, c.776 C>T (p.P259L) and c.818 A>G (p.Y273S)	−
2	32/F	4.5	−	2	+	24	2 missense mutations: exon 6, c.571 T>A (p.C191S) and c.578 G>A (p.S193N)	−
3	22/M	8	−	3	+	111	1 missense mutation: exon 3, c.221 T>C (p.M71T)	+
4	51/M	6.5	−	0	−	161	1 silent mutation: exon 1, c.171C>T (p.T57T)	+
5	35/M	3.5	+	4	−	72	1 silent mutation: exon 3, c.225T>C (p.A75A)	−
6	65/F	3	−	0	−	120	1 intronic mutation: exon 1, c.72+24G>A; and 1 silent mutation: exon 3, c.225T>C (p.A75A)	−

Abbreviations: HPF, high-power field; LVI, lymphovascular invasion; −, not present; +, present.

relevant criteria have not been well established. In previous studies, the incidence of malignancy in PBPGs was from 6.6% to 18% of all PBPGs.[13,17,18] Based on World Health Organization criteria, PGs are defined as malignant only when metastases are identified at the anatomic sites where ganglia are normally absent.[19] Predicting the malignant potential is an issue of great interest. Clinicopathologic characteristics and a scoring system have been suggested to predict malignant potential in pheochromocytomas of adrenal glands.[20] However, this scoring system has not been well accepted for PBPGs, and the biologic characteristics of PGs arising in the bladder may be different from the pheochromocytoma of the adrenal glands. A total of 37 cases of malignant PBPGs have been previously reported, as previously summarized by literature review.[21] In this review, malignant tumors occurred more frequently in younger males, whereas size was not correlated with the likelihood of metastasis.[21] In our study, we have also found that patients with SDHB-deficient tumors were younger than patients with SDHB-intact tumors (43 and 59 years, respectively; $P = .002$) at the time of diagnosis, but no preference in sex was identified ($P = .09$). A recent report showed a female predilection (8 of 11; 73%).[22] In contrast to the literature review, we found larger sizes of SDHB-deficient tumors compared with SDHB-intact tumors (means, 4.5 and 1.9 cm, respectively; $P < .001$). Our result is also consistent with the recent report by Gupta et al.[22] In the literature review, metastases in the pelvic lymph nodes were most common, followed by extrapelvic metastases, including mediastinum, lung, mesentery, liver, bone, and peritoneum.[21] Regarding our 3 cases, lymph nodes and bones were the metastatic sites.

We found that mitotic activity was higher for SDHB-deficient tumors compared with SDHB-intact tumors (2.6 and 0.1 mitoses per 10 high-power fields, respectively; $P = .002$). This result is consistent with previous research.[13] In our study, mitotic figures were very rare and difficult to find in SDHB-intact PGs, but occasional mitoses were identified in SDHB-deficient PGs, with 1 case of an SDHB-deficient PG exhibiting an atypical mitosis. We also found that the frequency of LVI was higher for SDHB-deficient tumors compared with SDHB-intact tumors (33% and 7%, respectively; $P = .02$). Reliable pathologic criteria have not been established for malignancy to date. In our study, we did not definitely identify any morphologic features that could be useful when predicting malignant PBPGs, because only 3 cases showed evidence of malignancy. However, large tumor size (6.2 and 2.1 cm, respectively; $P = .002$) and the presence of LVI (67% and 8%, respectively; $P = .03$) may be useful features in predicting aggressive biologic behavior of PBPGs.

SDH mutations have been found in approximately 30% of both head and neck, and thoracoabdominal PGs,[6,7] with *SDHB* mutations being detected more frequently in thoracoabdominal PGs compared with head and neck PGs.[6] Although *SDHB* mutations in PBPGs have been reported, the rate of *SDH* mutations in PBPGs has not been established to date. In 2 recent reports, *SDH* mutations were mainly *SDHB*, although *SDHA* mutations were also noted.[13] In accordance with these studies, our results also demonstrated that *SDHB* mutations predominated. *SDHB* mutations correlated with malignant behavior in both PGs and pheochromocytomas.[7–10] In our study, all 6 SDH-deficient cases that were successfully sequenced showed *SDHB* mutations. Until now, only 2 cases have shown malignant behavior and 4 exhibited a benign clinical course (Table 4). Two cases with malignant behavior have been followed up for an extended period of time (111 and 161 months, respectively), but 4 cases with indolent behavior have been followed up for a relatively short period of time, except for 1 case (21, 24, 72, and 120 months, respectively). Therefore, these 4 *SDHB*-mutated indolent PGs have to be followed up for an extended period of time in order to fully confirm whether they are truly benign or not, because in some cases, metastases can occur many years after the initial diagnosis.

Our study has several limitations. First, it was a retrospective, nationwide, multi-institutional study that evaluated the clinicopathologic characteristics of PBPGs and, as such, only paraffin-embedded tumor tissues were available for mutational analyses. This prevented a distinction between germ line and somatic mutations. However, cases with *SDHB* mutations are expected to be mostly germ line, because it is known that the vast majority of SDH-deficient PGs harbor germ line *SDH* mutations, with only rare reports of somatic *SDH* mutations.[23,24] Moreover, recent studies have also demonstrated that most cases with *SDHB* mutations were germ line.[13,25] Second, the follow-up period was relatively short (a mean of 41 months) compared with previous studies (means of 70 and 124 months, respectively).[13,22] This could explain the lower frequency of malignant PGs in our study (3 of 52; 5.8%) compared with the previous studies (2 of 11; 18%; and 5 of 11; 45%; respectively).[13,22] In one particular study, a single case of distant metastasis developed 10 years after diagnosis.[17] Therefore, longer follow-up periods are recommended to determine the exact

frequency of malignant PBPGs. Third, mutational analysis was not available for 1 SDHB-intact tumor that developed metastasis. Even though immunohistochemistry for SDHB provides a sensitive and specific method for identifying tumors with SDH mutation,[10] in this case, the tissue was not available for mutational analysis to document unequivocal mutation results. Nonetheless, in our study we used a relatively large cohort size of 52 cases with PBPGs, which were collected nationwide from multiple institutions in Korea; therefore, we believe that this study is representative of the general clinicopathologic characteristics of PBPGs.

In conclusion, PBPG is a very rare bladder tumor with 17% (9 of 52) incidence of SDH deficiency and 5.8% (3 of 52) incidence of malignancy. Younger age of the patient, large tumor size, higher number of mitoses, and presence of LVI and *SDHB* mutations are potential criteria to predict the malignant behavior of PBPGs.

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