Histol Histopathol (2014) 29: 1343-1354
DOI: 10.14670/HH-29.1343

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

The expression of succinate dehydrogenase in breast phyllodes tumor

Junjeong Choi[1], Do Hee Kim[2], WooHee Jung[2] and Ja Seung Koo[2]
[1]Department of Pathology, Yonsei University Wonju College of Medicine, Wonju and
[2]Department of Pathology, Yonsei University College of Medicine, Seoul, South Korea

Summary. The purpose of this study is to investigate the expression of succinate dehydrogenase (SDH)A, SDHB, and HIF-1α in phyllodes tumors and the association with clinic-pathologic factors. Using tissue microarray (TMA) for 206 phyllodes tumor cases, we performed immunohistochemical stains for SDHA, SDHB, and HIF-1α and analyzed their expression in regard to clinicopathologic parameters of each case. The cases were comprised of 156 benign, 34 borderline, and 16 malignant phyllodes tumors. The expression of stromal SDHA and epithelial- and stromal- SDHB increased as the tumor progressed from benign to malignant (P<0.001). There were five stromal SDHA-negative cases and 31 stromal SDHB-negative cases. SDHB negativity was associated with a lower histologic grade (P=0.054) and lower stromal atypia (P=0.048). Univariate analysis revealed that a shorter disease free survival (DFS) was associated with stromal SDHB high-positivity (P=0.013) and a shorter overall survival (OS) was associated with high-positivity of stromal SDHA and SDHB (P<0.001 and P<0.001, respectively). The multivariate Cox analysis with the variables stromal cellularity, stromal atypia, stromal mitosis, stromal overgrowth, tumor margin, stromal SDHA expression, and stromal SDHB expression revealed that stromal overgrowth was associated with a shorter DFS (hazard ratio: 24.78, 95% CI: 3.126-196.5, P=0.002) and a shorter OS (hazard ratio: 176.7, 95% CI: 8.466-3691, P=0.001). In conclusion, Tumor grade is positively correlated with SDHA and SDHB expression in the tumor stroma in phyllodes tumors of the breast. This result may be attributed to the increased metabolic demand in high grade tumors.

Key words: Breast, Phyllodes tumor, Succinate dehydrogenase

Offprint requests to: Ja Seung Koo, MD, PhD, Department of Pathology, Yonsei University College of Medicine, Severance Hospital, 50 Yonsei-ro, Seodaemun-gu, Seoul, 120-752 South Korea. e-mail: kjs1976@yuhs.ac

Introduction

Succinate dehydrogenase (SDH) is an enzyme complex located in the inner mitochondrial membrane and is responsible for cellular metabolism. As a member of the tricarboxylic acid (TCA) cycle and electron transport chain, it catalyzes the conversion of succinate into fumarate (Gottlieb and Tomlinson, 2005). The enzyme is comprised of four subunits (SDHA, SDHB, SDHC, and SDHD), among which SDHA and SDHB comprise the catalytic core (Sun et al., 2005). In addition to its important roles in the metabolic process, SDH is also known to be involved in tumorigenesis. A loss of function of SDH by a mutation of the SDH gene is observed in various tumors, most notably in pheochromocytoma (Astuti et al., 2001; van Nederveen et al., 2009), paragaglioma (Astuti et al., 2001; Baysal, 2003; van Nederveen et al., 2009; Burnichon et al., 2010), gastrointestinal stromal tumor (GIST) (Gill et al., 2010, 2011a; Gaal et al., 2011), and renal cell carcinoma (Gill et al., 2011b). Although evaluation of SDH gene mutations can be performed by gene sequencing, mutation-specific immunohistochemistry (IHC) has

successfully identified SDH gene mutations in several studies (van Nederveen et al., 2009; Burnichon et al., 2010; Korpershoek et al., 2011).

Phyllodes tumor is a rare disease entity, accounting for 0.3-1.5% of whole breast tumors. The tumor belongs to the fibroepithelial tumor group that includes tumors such as fibroadenoma. Phyllodes tumor shares many histologic features with fibroadenoma and even shows intratumoral histologic heterogeneity, which often causes problems in the differential diagnosis from fibroadenoma (Anderson et al., 2004; Lakhani et al., 2012). Although controversies exist in the histologic classification of phyllodes tumor, WHO classifies the tumor into benign, borderline, and malignant phyllodes (Lakhani et al., 2012). Higher grade tumors show aggressive clinical behavior such as increased tumor recurrence and distant metastasis. This study investigated the expression of SDHA, SDHB, and HIF-1α in phyllodes tumors and the association with clinic-pathologic factors.

Materials and methods

Patient selection

The study tissue was retrieved from the archives of the Department of Pathology at Severance Hospital. Patients diagnosed with phyllodes tumor who underwent surgical resection during the period of 1995 to 2010 were enrolled in the study. The study was approved by the Institutional Review Board of Yonsei University Severance Hospital. All tissues were fixed in 10% buffered formalin and embedded in paraffin. All archived hematoxylin and eosin (H&E)-stained slides for each case were reviewed by two pathologists (JS Koo and W Jung) to assess the histologic grade of phyllodes tumor. The histologic grading of phyllodes tumor was performed based on the WHO blue book (Lakhani et al., 2012) with H&E-stained slides of available sections. Age at diagnosis and clinical parameters such as tumor recurrence, distant metastasis, and survival were assessed.

Tissue microarray

After histologic review of the H&E-stained slides, a representative section from each case was selected, and the cores were punched out from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples. Considering selection bias, two 3-mm tissue cores from each case were acquired and transferred to a 6x5 recipient block.

Immunohistochemistry

All immunostaining was performed using formalin-fixed, paraffin-embedded tissue sections. Briefly, 5-μm-thick sections were obtained with a microtome, transferred onto adhesive slides, and dried at 62°C for 30 min. After incubation with primary antibody against SDHA (Abcam, Cambridge, UK, 1:100, 2E3GC12F-B2AE2), SDHB (Abcam, Cambridge, UK, 1:100, 21A11AE7) and HIF-1a (Biocare, Yorba Linda, CA, USA, 1:100, EP1215Y) immunodetection was performed with biotinylated anti-mouse immuno-globulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3-diaminobenzidine chromogen as the substrate. The primary antibody incubation step was omitted in the negative control. Slides were counterstained with Harris hematoxylin. All immunohistochemical markers were accessed by light microscopy. SDHA and SDHB IHC were assessed as negative when there was no expression (Barletta and Hornick, 2012) (Fig. 1). Granular positivity in the cytoplasm counted as positive expression, and the percentage of expression was assessed, categorizing the cases into 'low-positive' when there was 1-30% expression and high-positive when there was greater than 30% expression (Hameed et al., 2008). SDH immunohistochemistry was assessed by 2 pathologists with an internal control of peri-tumoral lymphocytes and endothelial cells. The negative cases were defined as total negative expression in this study and the potential discrepancy caused by the cut value of low vs. high expression set to 30% was minimized by using a reference slide showing 30% expression generated before the assessment. Thus there was minimal discrepancy in the assessment and interpretation between two cases and the third pathologist was consulted regarding discrepant cases. HIF-1α was assessed as positive when more than 10% of cells expressed the signal in the nucleus.

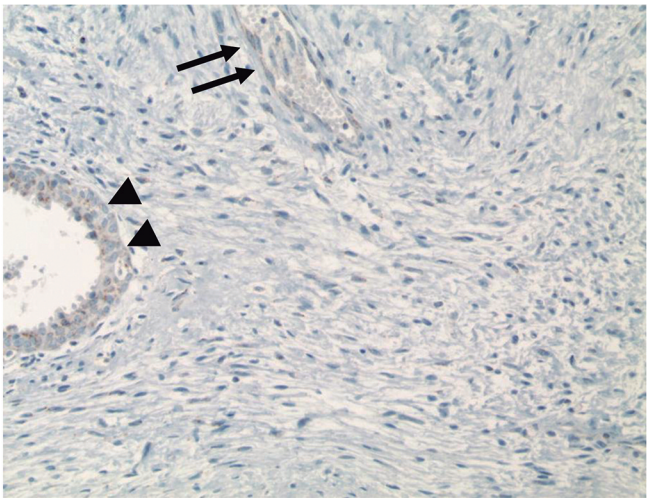

Fig. 1. Representative case without SDHA expression. Stromal component of PT is negative for SDHA, but endothelial cells (arrow) as internal positive control and epithelail component (arrow head) is positive for SDHA. x 200

Statistical analysis

Data were analyzed using SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA). For determination of statistical significance regarding various parameters, Student's t and Fisher's exact tests were used for continuous and categorical variables, respectively. For continuous variables, the Shaprio-Wilk test for normality was performed and the non-parametric Kruskal-Wallis test was employed for comparison when the null hypothesis of Shapiro-Wilk was rejected. Results were considered statistically significant when $P<0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence. Multivariate regression analysis was performed using a Cox proportional hazards model.

Results

Patient characteristics

The clinicopathological characteristics of patients are summarized in Table 1. A total of 206 phyllodes tumor cases were comprised of 156 benign, 34 borderline, and 16 malignant tumors. Patient age and tumor size were associated with a higher grade of phyllodes tumor ($P=0.013$ and $P=0.024$, respectively). The rate of treatment by mastectomy increased according to the grade of PT ($P<0.001$), higher tumor grade was also associated with tumor recurrence and distant metastasis ($P<0.001$). The site of distant metastasis for all eight cases was the lung.

Expression of SDHA and SDHB according to histologic grade of the phyllodes tumor

The expression of SDHA and SDHB in regard to the histologic grade of phyllodes tumors was assessed. Stromal expression of SDHA and epithelial and stromal expression of SDHB increased as the tumor grade progressed from benign to malignant (Figs. 2, 3, Table 2, $P<0.001$). We identified a zonal distribution of SDHA and SDHB expression in benign phyllodes tumors, as most expression was observed in the spindle cells of the periductal area (Fig. 4); however, this phenomenon was not observed in the borderline or malignant phyllodes

Table 1. Clinicopathologic characteristics of patients with phyllodes tumor.

Parameter	Number of Patients n=206 (%)	PT, Benign n=156 (%)	PT, Borderline n=34 (%)	PT, Malignant n=16 (%)	P-value
Age [years, median (range)]	41 (12-88)	40 (12-73)	45 (17-64)	45 (35-88)	0.013*
Tumor size [cm, median (range)]	3.1 (1.0-14.0)	3.0 (1.0-13.0)	3.5 (1.5-11.0)	4.9 (1.2-14.0)	0.024*
Surgery type					<0.001
Excision	192 (93.2)	149 (95.5)	33 (97.1)	10 (62.5)	
Mastectomy	14 (6.8)	7 (4.5)	1 (2.9)	6 (37.5)	
Stromal cellularity					<0.001
Mild	124 (60.2)	122 (78.2)	2 (5.9)	0 (0.0)	
Moderate	68 (33.0)	34 (21.8)	27 (79.4)	7 (43.8)	
Marked	14 (6.8)	0 (0.0)	5 (14.7)	9 (56.3)	
Stromal atypia					<0.001
Mild	161 (78.2)	154 (98.7)	7 (20.6)	0 (0.0)	
Moderate	34 (16.5)	2 (1.3)	24 (70.6)	8 (50.0)	
Marked	11 (5.3)	0 (0.0)	3 (8.8)	8 (50.0)	
Stromal mitosis					<0.001
0-3 / 10 HPFs	160 (77.7)	156 (100.0)	4 (11.8)	0 (0.0)	
4-9 / 10 HPFs	35 (17.0)	0 (0.0)	30 (88.2)	5 (31.3)	
>10 / 10 HPFs	11 (5.3)	0 (0.0)	0 (0.0)	11 (68.8)	
Stromal overgrowth					<0.001
Absent	188 (91.3)	156 (100.0)	30 (88.2)	2 (12.5)	
Present	18 (8.7)	0 (0.0)	4 (11.8)	14 (87.5)	
Tumor margin					<0.001
Circumscribed	185 (89.8)	153 (98.1)	26 (76.5)	6 (37.5)	
Infiltrative	21 (10.2)	3 (1.9)	8 (23.5)	10 (62.5)	
Tumor recurrence	18 (8.7)	5 (3.2)	6 (17.6)	7 (43.8)	<0.001
Distance metastasis	8 (3.9)	0 (0.0)	1 (2.9)	7 (43.8)	<0.001
Radiation therapy	26 (12.6)	19 (12.2)	5 (14.7)	2 (12.5)	0.818
Duration of follow-up (months, mean ± SD)	74.4±48.1	82.2±48.3	58.1±38.7	31.1±31.6	<0.001

PT, phyllodes tumor; HPFs, high-power fields. * p-value was calculated by Kruskall-Wallis test.

tumor cases.

Expression status of SDHA and SDHB according to HIF-1α status

The expression of SDHA and SDHB with regard to HIF-1α expression was assessed (Table 3). There was a tendency for a positive association between stromal SDHB expression with stromal HIF-1α expression, although this finding was not statistically significant. (P=0.062).

Clinicopathologic features of phyllodes tumor with SDHA and/or SDHB negativity in the stromal component

There were two epithelial SDHA-negative cases,

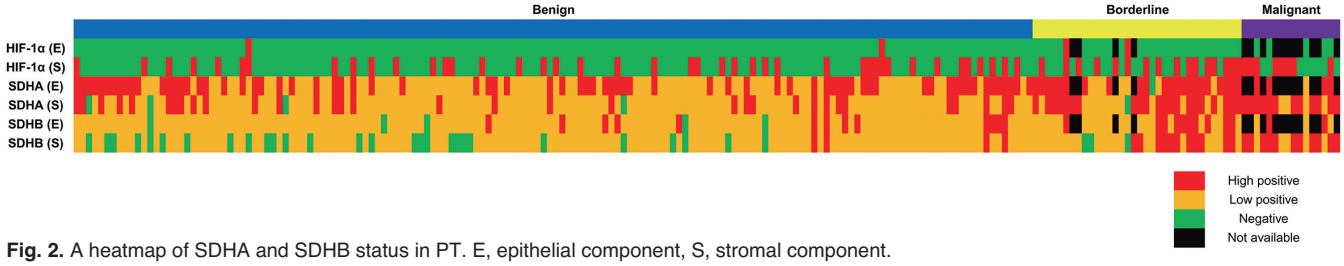

Fig. 2. A heatmap of SDHA and SDHB status in PT. E, epithelial component, S, stromal component.

Table 2. Expression of SDHA, SDHB, and HIF-1α according to the histologic grade of phyllodes tumor.

Parameter	No. of Patients n=206 (%)	PT, Benign n=156 (%)	PT, Borderline n=34 (%)	PT, Malignant n=16 (%)	P-value
SDHA(E)* [%,median (range)]	30 (0-100)	20 (5-100)	30 (0-60)	30 (20-50)	0.094 †
SDHA(S) [%,median (range)]	15 (0-70)	10 (0-70)	40 (0-60)	45 (10-70)	<0.001 †
SDHB(E)* [%,median (range)]	10 (0-40)	10 (0-40)	20 (5-40)	20 (10-30)	<0.001 †
SDHB(S) [%,median (range)]	5 (0-70)	5 (0-40)	20 (0-70)	30 (5-60)	<0.001 †
HIF-1α (E)*					0.201
Negative	186 (97.9)	154 (98.7)	27 (93.1)	5 (100.0)	
Positive	4 (2.1)	2 (1.3)	2 (6.9)	0 (0.0)	
HIF-1α (S)					0.011
Negative	139 (67.5)	113 (72.4)	18 (52.9)	8 (50.0)	
Positive	67 (32.5)	43 (27.6)	16 (47.1)	8 (50.0)	

PT, phyllodes tumor. *14 cases without an epithelial component were excluded. † p-value was calculated by Kruskall-Wallis test.

Table 3. Expression status of SDHA and SDHB according to HIF-1α status.

Parameter	Epithelial HIF-1α*			Stromal HIF-1α		
	Negative n=186 (%)	Positive n=4 (%)	P-value†	Negative n=139 (%)	Positive n=67 (%)	P-value†
SDHA (E)*			0.588			1.262
Negative	1 (0.5)	0 (0.0)		2 (1.5)	0 (0.0)	
Low	87 (46.8)	3 (75.0)		63 (47.7)	27 (45.8)	
High	98 (52.7)	1 (25.0)		67 (50.8)	32 (54.2)	
SDHA (S)			0.586			0.262
Negative	4 (2.2)	1 (25.0)		4 (2.9)	1 (1.5)	
Low	129 (69.4)	2 (50.0)		95 (68.3)	40 (59.7)	
High	53 (28.5)	1 (25.0)		40 (28.8)	26 (38.8)	
SDHB (E)*			0.892			0.102
Negative	5 (2.7)	0 (0.0)		4 (3.1)	1 (1.7)	
Low	157 (84.4)	3 (75.0)		114 (87.0)	46 (78.0)	
High	24 (12.9)	1 (25.0)		13 (9.9)	12 (20.3)	
SDHB (S)			0.168			0.062
Negative	29 (14.4)	2 (50.0)		24 (17.3)	7 (10.4)	
Low	141 (75.8)	2 (50.0)		102 (73.4)	47 (70.1)	
High	16 (8.6)	0 (0.0)		13 (9.4)	13 (19.4)	

*14 cases without an epithelial component were excluded. † p-value is corrected by Bonferroni correction method.

five epithelial SDHB-negative cases, five stromal SDHA-negative cases, and 31 stromal SDHB-negative cases. No cases for SDHA and 4 for SDHB were negative for both epithelial and stromal components. Stromal expression of SDHA and SDHB were assessed and 175(85.05%) SDHA(+)/SDHB(+), 26(12.6%), SDHA (+)/SDHB(-), 0(0.0%) SDHA(-)/SDHB(+) and 5(2.4%) SDHA (-)/SDHB(-) cases were found. Stromal SDHA- and/or SDHB-negative cases were analyzed with clinicopathologic features, which revealed that stromal SDHA negativity was associated with younger age (P=0.002). Stromal SDHB negativity was associated with younger age (P<0.001), smaller tumor size (P<0.001), lower stromal atypia (P=0.048) when compared to stromal SDHB positive cases. In addition, there was a tendency for lower histologic grade (P=0.054) in the group with stromal SDHB negativity (Table 4). No SDH-related tumors such as phoechromocytoma or GIST in patients with SHDA and/or SDHB negative tumors were found.

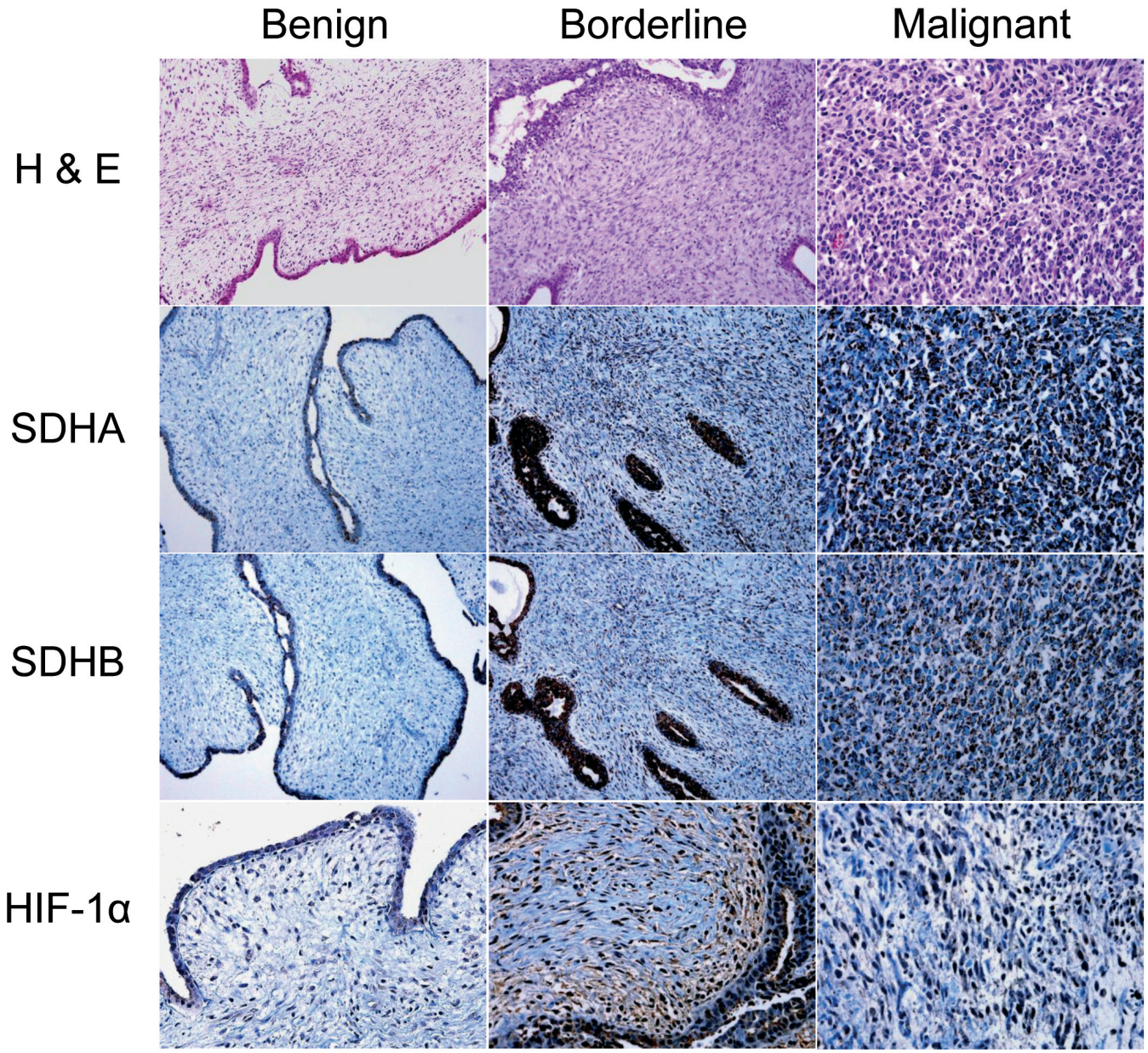

Fig. 3. Immunohistochemical expression of SDHA, SDHB, and HIF-1α according to phyllodes tumor grade. The expression of stromal SDHA, stromal SDHB, and epithelial SDHB increased as the tumor progressed from benign to malignant. x 200

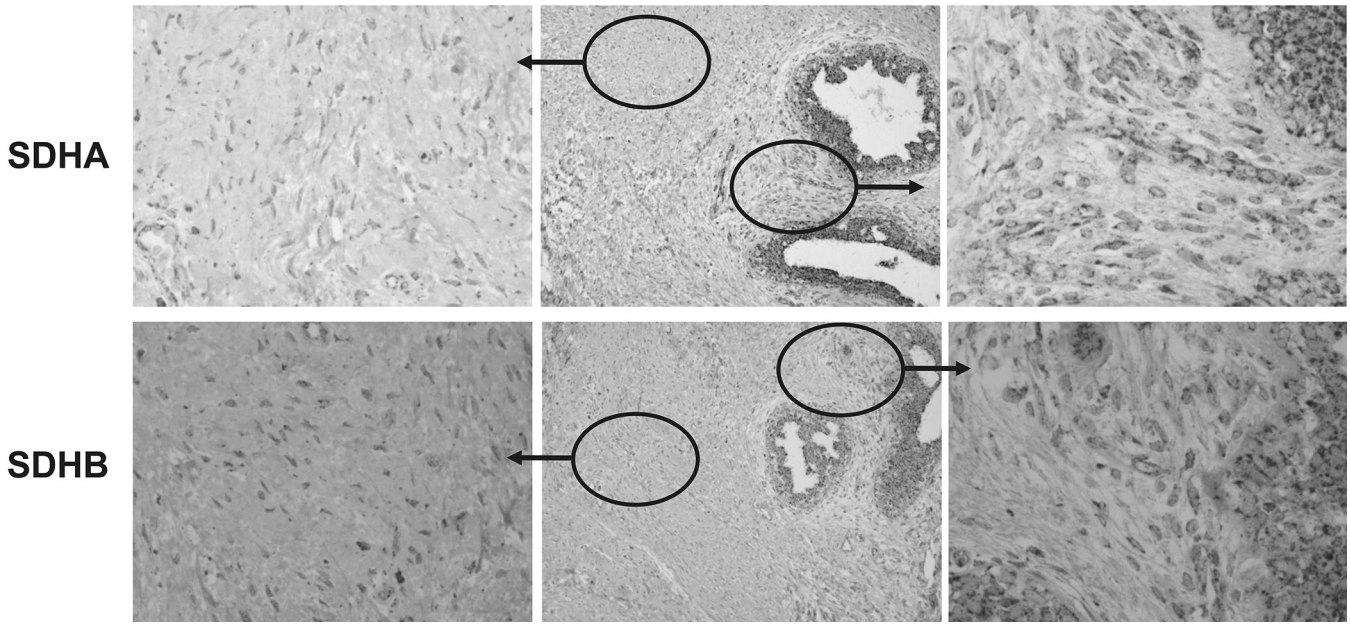

Fig. 4. Expression pattern of SDHA and SDHB in benign phyllodes tumors. SDHA and SDHB are mainly expressed in the periductal spindle cells of the tumor and not in the stromal cells apart from the ductal structure. Right and left, 400; center, x 200

Table 4. Clinicopathologic features of phyllodes tumors showing SDHA and/or SDHB negativity in the stromal component.

Parameter	SDHA			SDHB		
	Negative n=5 (%)	Positive n=201 (%)	P-value*	Negative n=31 (%)	Positive n=175 (%)	P-value*
Age [years, median (range)]	39 (12-73)	45 (17-88)	0.002†	40 (12-73)	47 (32-88)	<0.001†
Tumor size [cm, median (range)]	3.0 (1.0-12.9)	3.5 (1.2-14.0)	0.128†	3.0 (1.0-12.9)	4.9 (1.5-14.0)	<0.001†
Histologic grade			1.598			0.054
Benign	4 (80.0)	152 (75.6)		28 (90.3)	128 (73.1)	
Borderline	1 (20.0)	33 (16.4)		3 (9.7)	31 (17.7)	
Malignant	0 (0.0)	16 (8.0)		0 (0.0)	16 (9.1)	
Stromal cellularity			1.618			0.486
Mild	3 (60.0)	121 (60.2)		21 (67.7)	103 (58.9)	
Moderate	2 (40.0)	66 (32.8)		10 (32.3)	58 (33.1)	
Marked	0 (0.0)	14 (7.0)		0 (0.0)	14 (8.0)	
Stromal atypia			0.978			0.048
Mild	5 (100.0)	156 (77.6)		30 (96.8)	131 (74.9)	
Moderate	0 (0.0)	34 (16.9)		1 (3.2)	33 (18.9)	
Marked	0 (0.0)	11 (5.5)		0 (0.0)	11 (6.3)	
Stromal mitosis			0.958			0.126
0-3 / 10 HPFs	5 (100.0)	155 (77.1)		29 (93.5)	131 (74.9)	
4-9 / 10 HPFs	0 (0.0)	35 (17.4)		2 (6.5)	33 (18.9)	
>10 / 10 HPFs	0 (0.0)	11 (5.5)		0 (0.0)	11 (6.3)	
Stromal overgrowth			2.000			0.162
Absent	5 (100.0)	183 (91.0)		31 (100.0)	157 (89.7)	
Present	0 (0.0)	18 (9.0)		0 (0.0)	18 (10.3)	
Tumor margin			2.000			0.424
Circumscribed	5 (100.0)	180 (89.6)		30 (96.8)	155 (88.6)	
Infiltrative	0 (0.0)	21 (10.4)		1 (3.2)	20 (11.4)	
Tumor recurrence	0 (0.0)	18 (9.0)	2.000	2 (6.5)	16 (9.1)	2.000
Distance metastasis	0 (0.0)	8 (4.0)	2.000	0 (0.0)	8 (4.6)	1.218

* P-value is corrected by Bonferroni method.

Correlations between clinicopathologic parameters and SDH expression

A high-positive expression of epithelial SDHB and stromal SDHA/SDHB was associated with a higher histologic grade, higher stromal atypia and increased stromal mitosis (P<0.05). A high-positive expression of stromal SDHA and SDHB was related to stromal overgrowth and infiltrative tumor margin (P<0.05). High-positive stromal SDHB expression was related to

Table 5. Correlations between clinicopathologic parameters and SDHA/SDHB expression.

Parameter	SDHA						SDHB					
	Epithelial component*			Stromal component			Epithelial component*			Stromal component		
	(-)/Low n=91 (%)	High n=99 (%)	P-value†	(-)/Low n=140 (%)	High n=66 (%)	P-value†	(-)/Low n=165 (%)	High n=25 (%)	P-value†	(-)/Low n=180 (%)	High n=26 (%)	P-value†
Histologic grade			0.056			<0.001			<0.001			<0.001
Benign	82 (90.1)	73 (73.7)		123 (87.9)	33 (50.0)		143 (86.7)	13 (52.0)		152 (84.4)	4 (15.4)	
Borderline	8 (8.8)	22 (22.2)		13 (9.3)	21 (31.8)		19 (11.5)	10 (40.0)		23 (12.8)	11 (42.3)	
Malignant	1 (1.1)	4 (4.0)		4 (2.9)	12 (18.2)		3 (1.8)	2 (8.0)		5 (2.8)	11 (42.3)	
Stromal cellularity			0.172			<0.001			0.060			<0.001
Mild	66 (72.5)	57 (57.6)		99 (70.7)	25 (37.9)		114 (69.1)	10 (40.0)		121 (67.2)	3 (11.5)	
Moderate	24 (26.4)	36 (36.4)		40 (28.6)	28 (42.4)		46 (27.9)	13 (52.0)		55 (30.6)	13 (50.0)	
Marked	1 (1.1)	6 (6.1)		1 (0.7)	13 (19.7)		5 (3.0)	2 (8.0)		4 (2.2)	10 (38.5)	
Stromal atypia			0.160			<0.001			<0.001			<0.001
Mild	83 (91.2)	77 (77.8)		126 (90.0)	35 (53.0)		147 (89.1)	13 (52.0)		157 (87.2)	4 (15.4)	
Moderate	7 (7.7)	19 (19.2)		12 (8.6)	22 (33.3)		17 (10.3)	9 (36.0)		20 (11.1)	14 (53.8)	
Marked	1 (1.1)	3 (3.0)		2 (1.4)	9 (13.6)		1 (0.6)	3 (12.0)		3 (1.7)	8 (30.8)	
Stromal mitosis			0.080			<0.001			<0.001			<0.001
0-3 / 10 HPFs	83 (91.2)	76 (76.8)		125 (89.3)	35 (53.0)		145 (87.9)	15 (60.0)		155 (86.1)	5 (19.2)	
4-9 / 10 HPFs	8 (8.8)	21 (21.2)		13 (9.3)	22 (33.3)		19 (11.5)	9 (36.0)		22 (12.2)	13 (50.0)	
>10 / 10 HPFs	0 (0.0)	2 (2.0)		2 (1.4)	9 (13.6)		1 (0.6)	1 (4.0)		3 (1.7)	8 (30.8)	
Stromal overgrowth			0.488			<0.001			0.340			<0.001
Absent	91 (100.0)	95 (96.0)		137 (97.9)	51 (77.3)		163 (98.8)	23 (92.0)		173 (96.1)	15 (57.7)	
Present	0 (0.0)	4 (4.0)		3 (2.1)	15 (22.7)		2 (1.2)	2 (8.0)		7 (3.9)	11 (42.3)	
Tumor margin			0.044			0.020			1.608			0.004
Circumscribed	89 (97.8)	87 (87.9)		132 (94.3)	53 (80.3)		154 (93.3)	22 (88.0)		167 (92.8)	18 (69.2)	
Infiltrative	2 (2.2)	12 (12.1)		8 (5.7)	13 (19.7)		11 (6.7)	3 (12.0)		13 (7.2)	8 (30.8)	
Tumor recurrence	6 (6.6)	6 (6.1)	4.000	10 (7.1)	8 (12.1)	1.164	9 (5.5)	3 (12.0)	0.792	13 (7.2)	5 (19.2)	0.232
Distance metastasis	0 (0.0)	2 (2.0)	1.992	3 (2.1)	5 (7.6)	0.456	0 (0.0)	2 (8.0)	0.068	4 (2.2)	4 (15.4)	0.040

*14 cases without an epithelial component were excluded. † p-value is corrected by Bonferroni correction method.

Table 6. Univariate analysis of the impact of expression of SDHA and SDHB on prognosis by the log-rank test.

Parameter	Total number/recurrence/death	Disease-free survival		Overall survival	
		Median survival (95% CI) months	P-value	Median survival (95% CI) months	P-value
SDHA (E)*			0.999		n/a
Negative / Low	91 / 6 / 0	150 (142–157)		n/a	
High	99 / 6 / 2	171 (163–180)		n/a	
SDHA (S)			0.189		<0.001
Negative / Low	140 / 10 / 1	164 (157–172)		175 (173–178)	
High	66 / 8 / 7	161 (147–175)		163 (149–177)	
SDHB (E)*			0.120		n/a
Negative / Low	165 / 9 / 2	173 (166–179)		n/a	
High	25 / 3 / 0	83 (71–95)		n/a	
SDHB (S)			0.013		<0.001
Negative / Low	180 / 13 / 4	170 (163–176)		179 (175–182)	
High	26 / 5 / 4	108 (87–129)		114 (96–132)	

*14 cases without an epithelial component were excluded.

distant metastasis (P=0.040, Table 5).

The impact of expression of SDHA and SDHB on prognosis

Univariate analysis of SDHA and SDHB expression with patient prognosis revealed that a shorter disease free survival (DFS) was associated with stromal SDHB high-positivity (P=0.013), and a shorter overall survival (OS) was associated with stromal SDHA high-positivity (P<0.001) and stromal SDHB high-positivity (P<0.001) (Fig. 5, Table 6). A multivariate Cox analysis with the variables of stromal cellularity, stromal atypia, stromal mitosis, stromal overgrowth, tumor margin, stromal SDHA expression, and stromal SDHB expression revealed that stromal overgrowth was associated with a shorter DFS (hazard ratio: 24.78, 95% CI: 3.126-196.5, P=0.002) and a shorter OS (hazard ratio: 176.7, 95% CI:

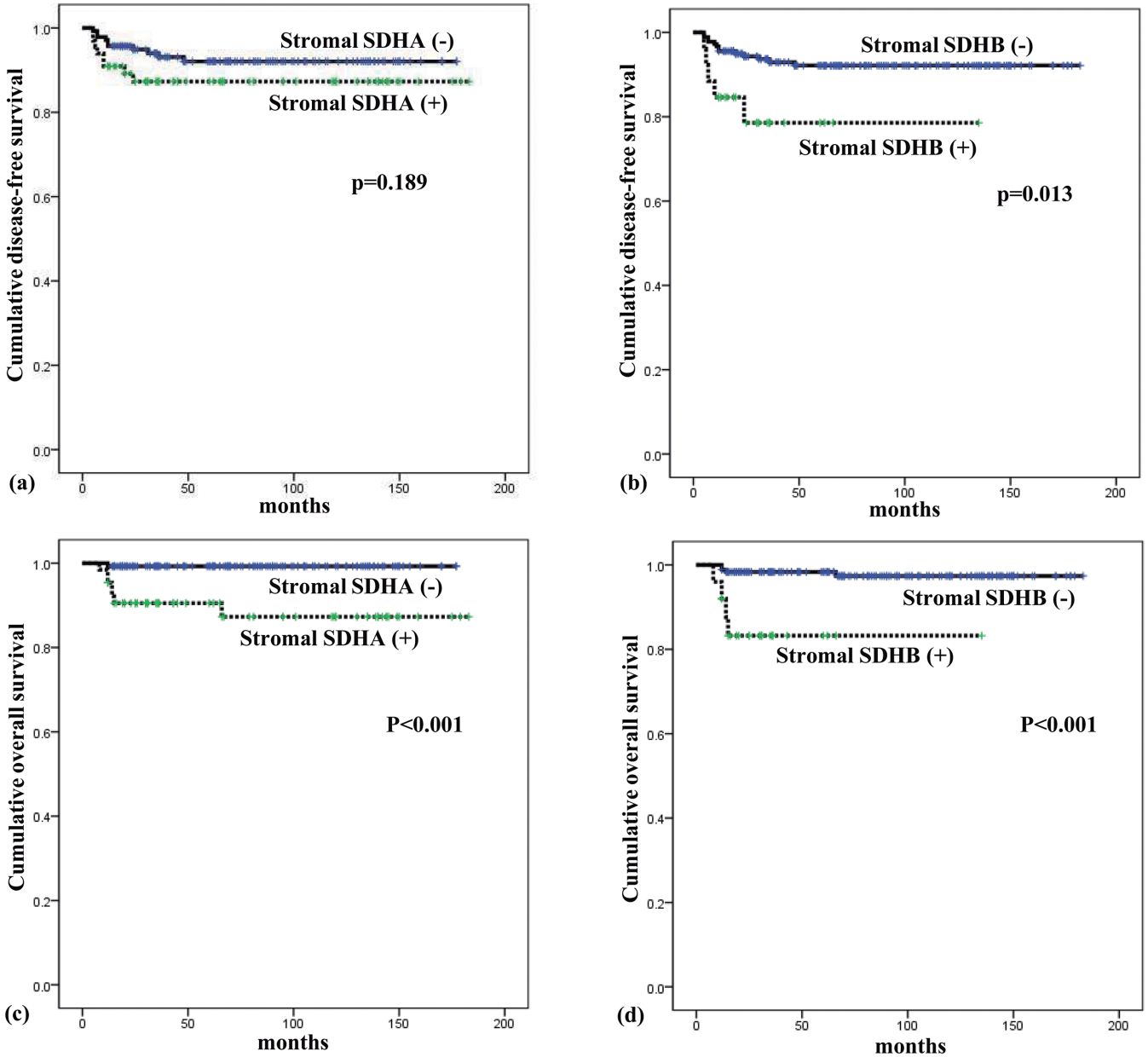

Fig. 5. The impacts of stromal SDHA **(a, c)** and SDHB **(b, d)** expression on disease-free survival **(a, b)** and overall survival **(c, d)**.

Table 7. Independent prognostic factors for disease-free survival and overall survival by multivariate analysis.

Parameter	Disease-free survival			Overall survival		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Stromal cellularity			0.869			0.412
Mild vs. Moderate/marked	1.252	0.086–18.17		6.168	0.080–475.1	
Stromal atypia			0.734			0.477
Mild vs. Moderate/marked	0.749	0.141–3.966		0.550	0.106–2.864	
Stromal mitosis			0.934			0.552
0-3/10HPFs vs. >4/10HPFs	0.889	0.056–14.22		0.260	0.003–21.92	
Stromal overgrowth			0.002			0.001
Absent vs. present	24.78	3.126–196.5		176.7	8.466–3691	
Tumor margin			0.967			0.470
Circumscribed vs. Infiltrative	1.030	0.256–4.139		0.555	0.112–2.742	
Stromal SDHA			0.402			0.689
Negative/Low vs. High	0.474	0.086–2.716		1.840	0.093–36.36	
Stromal SDHB			0.897			0.588
Negative/Low vs. High	0.880	0.126–6.125		0.560	0.069–4.563	

8.466-3691, P=0.001, Table 7).

Discussion

In this study, immunohistochemistry in phyllodes tumors revealed an increased expression of stromal SDHA and SDHB and epithelial SDHB in higher grade tumors. As there is no reported study regarding SDH expression in phyllodes tumors of the breast, the discussion of results in the context of previously reported studies is limited. The main biological function of SDH is the oxidation of succinate to fumarate and is related to the electron transporter (Gottlieb and Tomlinson, 2005). Accordingly, SDH is closely related to mitochondrial metabolism, which may suggest increased mitochondrial metabolism in the stromal component of high grade phyllodes tumors. SDH was shown to have a tumor suppressor function in several studies (Astuti et al., 2001; Baysal, 2003; Burnichon et al., 2010), and a loss of SDH gene function has previously been identified in paraganglioma, pheochromocytoma, and GIST. Previous authors posited that a deficiency of SDH might be related to tumorigenesis (Astuti et al., 2001; Baysal, 2003; Burnichon et al., 2010; Doyle et al., 2012; Gaal et al., 2011; Gill et al., 2010, 2011a), however, considering the results of the present study, this notion cannot be applied to breast phyllodes tumors. The mechanisms of SDH loss that lead to tumor formation are explained by the fact that a loss of SDH results in a hypoxia response under normoxic conditions (pseudohypoxia) through HIF-α (Baysal, 2003; Pollard et al., 2003, 2005; Burnichon et al., 2010). A high expression of HIF-1α in SDH-deficient tumors was reported in previous studies (Gimenez-Roqueplo et al., 2003; Pollard et al., 2005; Burnichon et al., 2010), and the authors raised the possibility that a loss of SDH leads to the accumulation of succinate, which stabilizes HIF-1α. In contrast with this SDH-deficiency tumorigenesis theory, in this study, HIF-1α expression in the stromal component was positively correlated with SDHA and SDHB expression and tumor grade. A possible explanation for this discrepancy may be the alternative methods of HIF-1α activation or stabilization in addition to SDH-deficiency. In addition to SDH-deficiency, oncogenic activation such as c-myc, growth factors such as IGF-1, and hypoxia were reported to be related to HIF-1α activation/stabilization (Kim et al., 2007). Kuijper et al. reported that stromal HIF-1α was positively correlated with the grade of phyllodes tumor (Kuijper et al., 2005), consistent with the results of the present study, and they suggested p53 inactivation as a potential mechanism for upregulation of HIF-1α.

A positive correlation between the expression of glycolysis-related proteins such as Glut-1 and CAIX in the stromal component and phyllodes tumor grade has been reported (Kwon et al., 2013), which suggests glycolysis is increased in the stromal components of tumors. The metabolism of malignant tumors is generally explained by the Warburg effect theory, in which a metabolic shift from mitochondrial oxidative phosphorylation through the TCA cycle to glycolysis occurs in tumors (Warburg, 1956). As the tumor progresses to a higher grade, metabolic activity is increased in phyllodes tumors. When the Warburg theory was first introduced, enhanced glycolysis by the tumor was thought to irreversibly damage mitochondrial function; however, oxidative metabolism was observed in many tumor cell types in following studies (Pedersen, 1978). Moreover, different types of tumor cells seem to use different predominant energy metabolisms, including glycolysis or oxidative phosphorylation (Moreno-Sanchez et al., 2007). The results of the present study suggest phyllodes tumors of the breast use both

mitochondrial pathway and TCA cycle for metabolism. Proliferation of the stromal component is increased as the tumor progresses to a higher grade, and the metabolic demand of the tumor increases accordingly, which is reflected by an increase in SDH expression in high grade tumors. Thus, PTs demonstrate more metabolic activity such as glycolysis, mitochondrial metabolism, and TCA cycle as the metabolic demand increases. In the univariate analysis of this study, high positivity of SDHA and SDHB in the stromal component was related with poor prognosis. This is aligned with a previous study showing that a high expression of glycolysis-related protein was related with poor prognosis (Younes et al., 1995, 1997; Stackhouse et al., 2005). This can be understood in that the high metabolic status may be related with tumor prognosis. However, further studies investigating this hypothesis should be performed as the result may simply reflect the association of SDH expression with other adverse histologic parameters, given that it was not statistically significant in the multivariate analysis. Another unique finding in this study is the zonal pattern of SDH expression in benign phyllodes tumors, showing a higher expression in the periductal stroma in which increased stromal cellularity and higher mitotic activity were reported (Tavassoli et al., 2003). As these histologic features suggest higher proliferative activity in this sub-compartment, this result also suggests a correlation between the expression of SDH and the metabolic demand of tumor cells.

There were a small number of stromal SDH-negative cases: only 5% of cases were SDHA negative and 31% of cases were SDHB negative. The negative results of SDH IHC need to be validated by mutation analysis to ascertain if they truly reflect the SDH gene mutation. Although IHC successfully identified SDHA and SDHB mutations in previous studies (van Nederveen et al., 2009; Burnichon et al., 2010; Korpershoek et al., 2011), it is still possible that a portion of PTs cases without expression of SDH by IHC actually harbor a SDH mutation. We applied strict categories to define negative expression of SDHA and SDHB, including counting cases as negative only if the entire tumor tissue evaluated did not show any expression, as previously suggested (Barletta and Hornick, 2012), and acquiring two cores from each case to prevent selection bias. Still, the results need to be interpreted with caution, as there remains a chance that the acquired tissue from TMA may not reflect whole tumor characteristics, as well as the assessment of SDH expression in normal tissue since TMA cores did not include peritumoral normal tissue.

Regarding the histologic features of the tumor with regard to SDH expression, there was no histologic difference between SDH mutation-positive and -negative cases in paraganglioma and/or pheochromocytoma (van Nederveen et al., 2009). In contrast, distinct histological differences were identified in the SDHB-negative group in GIST (Gill et al., 2010, 2011a) and renal cell carcinoma (Gill et al., 2011b). There was no distinct histologic feature in the SDH-negative tumors in the present study, except that stromal SDHB-negative cases showed a lower histologic grade and lower stromal cell atypia.

Diagnostically, it is not certain if there is any clinical implication in the finding that increased expression of SDHA and SDHB in the stromal component was positively correlated with the tumor grade. A potential applicable diagnostic application includes using these makers in a differential diagnosis of fibroepithelial tumors, especially for fibroadenoma and PTs. These two disease entities show significantly overlapped histology and no specific biomarker separating them is known, which causes diagnostic difficulties, especially in the diagnosis with limited amount of tissue, such as core biopsy. Thus, further study on the differential expression of SDH between fibroadenoma and PTs should be performed. Another clinical implication of this study is that the results may provide targets for therapeutic intervention. Several preclinical studies targeting metabolism-related markers in different types of tumors are being conducted, and inhibitors of HIF-1α (Chang et al., 2003; Yeo et al., 2003), Glut1 (Aft et al., 2002; Mohanti et al., 1996) CAIX (Vullo et al., 2003), and MCT4 (Gallagher et al., 2007) have been shown to suppress tumor growth. Accordingly, the feasibility of applying metabolic inhibitor such as HIF-1α inhibitor to primary and/or metastatic malignant phyllodes tumors needs to be considered, and further study should be performed given that there is a limited choice of medical treatment for PT at this moment.

In conclusion, tumor grade is positively correlated with SDHA and SDHB expression in the tumor stroma in phyllodes tumors of the breast and this result may be attributed to the increased metabolic demand in high grade tumors.

Acknowledgement. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A1002886).

Disclosure of Potential Conflicts of Interest: No potential conflicts of interest were disclosed.

References

Aft R.L., Zhang F.W. and Gius D. (2002). Evaluation of 2-deoxy-D-glucose as a chemotherapeutic agent: mechanism of cell death. Br. J. Cancer 87, 805-812.

Anderson B.O., Lawton T.J., Lehman C.D. and Moe R.E. (2004). Phyllodes tumor. In: Disease of the breast. 3rd ed. Harris J.R., Lippman M.E., Morrow M. and Osborne K.C. (eds). Lippincott & Wilkins, Philadelphia. pp 991-1006.

Astuti D., Latif F., Dallol A., Dahia P.L., Douglas F., George E., Skoldberg F., Husebye E.S., Eng C. and Maher E.R. (2001). Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am. J. Hum. Genet. 69, 49-54.

Barletta J.A. and Hornick J.L. (2012). Succinate dehydrogenase-deficient tumors: diagnostic advances and clinical implications. Adv. Anat. Pathol. 19, 193-203.

Baysal B.E. (2003). On the association of succinate dehydrogenase mutations with hereditary paraganglioma. Trends Endocrinol. Metab. 14, 453-459.

Burnichon N., Briere J.J., Libe R., Vescovo L., Riviere J., Tissier F., Jouanno E., Jeunemaitre X., Benit P., Tzagoloff A., Rustin P., Bertherat J., Favier J. and Gimenez-Roqueplo A.P. (2010). SDHA is a tumor suppressor gene causing paraganglioma. Hum. Mol. Genet. 19, 3011-3020.

Chang H., Shyu K.G., Lee C.C., Tsai S.C., Wang B.W., Hsien Lee Y. and Lin S. (2003). GL331 inhibits HIF-1alpha expression in a lung cancer model. Biochem. Biophys. Res. Commun. 302, 95-100.

Doyle L.A., Nelson D., Heinrich M.C., Corless C.L. and Hornick J.L. (2012). Loss of succinate dehydrogenase subunit B (SDHB) expression is limited to a distinctive subset of gastric wild-type gastrointestinal stromal tumours: a comprehensive genotype-phenotype correlation study. Histopathology 61, 801-809.

Gaal J., Stratakis C.A., Carney J.A., Ball E.R., Korpershoek E., Lodish M.B., Levy I., Xekouki P., van Nederveen F.H., den Bakker M.A., O'Sullivan M., Dinjens W.N. and de Krijger R.R. (2011). SDHB immunohistochemistry: a useful tool in the diagnosis of Carney-Stratakis and Carney triad gastrointestinal stromal tumors. Mod. Pathol. 24, 147-151.

Gallagher S.M., Castorino J.J., Wang D. and Philp N.J. (2007). Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. Cancer Res. 67, 4182-4189.

Gill A.J., Chou A., Vilain R., Clarkson A., Lui M., Jin R., Tobias V., Samra J., Goldstein D., Smith C., Sioson L., Parker N., Smith R.C., Sywak M., Sidhu S.B., Wyatt J.M., Robinson B.G., Eckstein R.P., Benn D.E. and Clifton-Bligh R.J. (2010). Immunohistochemistry for SDHB divides gastrointestinal stromal tumors (GISTs) into 2 distinct types. Am. J. Surg. Pathol. 34, 636-644.

Gill A.J., Chou A., Vilain R.E. and Clifton-Bligh R.J. (2011a). "Pediatric-type" gastrointestinal stromal tumors are SDHB negative ("type 2") GISTs. Am. J. Surg. Pathol. 35, 1245-1247; author reply 1247-1248.

Gill A.J., Pachter N.S., Chou A., Young B., Clarkson A., Tucker K.M., Winship I.M., Earls P., Benn D.E., Robinson B.G., Fleming S. and Clifton-Bligh R.J. (2011b). Renal tumors associated with germline SDHB mutation show distinctive morphology. Am. J. Surg. Pathol. 35, 1578-1585.

Gimenez-Roqueplo A.P., Favier J., Rustin P., Rieubland C., Crespin M., Nau V., Khau Van Kien P., Corvol P., Plouin P.F. and Jeunemaitre X. (2003). Mutations in the SDHB gene are associated with extra-adrenal and/or malignant phaeochromocytomas. Cancer Res. 63, 5615-5621.

Gottlieb E. and Tomlinson I.P. (2005). Mitochondrial tumour suppressors: a genetic and biochemical update. Nat. Rev. Cancer 5, 857-866.

Hameed O., Adams A.L., Baker A.C., Balmer N.E., Bell W.C., Burford H.N., Chhieng D.C., Jhala N.C., Klein M.J. and Winokur T. (2008). Using a higher cutoff for the percentage of HER2+ cells decreases interobserver variability in the interpretation of HER2 immunohistochemical analysis. Am. J. Clin. Pathol. 130, 425-427.

Kim J.W., Gao P. and Dang C.V. (2007). Effects of hypoxia on tumor metabolism. Cancer Metastasis Rev. 26, 291-298.

Korpershoek E., Favier J., Gaal J., Burnichon N., van Gessel B., Oudijk L., Badoual C., Gadessaud N., Venisse A., Bayley J.P., van Dooren M.F., de Herder W.W., Tissier F., Plouin P.F., van Nederveen F.H., Dinjens W.N., Gimenez-Roqueplo A.P. and de Krijger R.R. (2011). SDHA immunohistochemistry detects germline SDHA gene mutations in apparently sporadic paragangliomas and pheochromocytomas. J. Clin. Endocrinol. Metab. 96, E1472-1476.

Kuijper A., van der Groep P., van der Wall E. and van Diest P.J. (2005). Expression of hypoxia-inducible factor 1 alpha and its downstream targets in fibroepithelial tumors of the breast. Breast Cancer Res. 7, R808-818.

Kwon J.E., Jung W.H. and Koo J.S. (2013). The expression of metabolism-related proteins in phyllodes tumors. Tumour Biol. 34, 115-124.

Lakhani S.R., Ellis I.O., Schnitt S.J., Tan P.H. and Van de Vijver M.J. (2012). WHO Classification of Tumors of the Breast. WHO press. Geneva.

Mohanti B.K., Rath G.K., Anantha N., Kannan V., Das B.S., Chandramouli B.A., Banerjee A.K., Das S., Jena A., Ravichandran R., Sahi U.P., Kumar R., Kapoor N., Kalia V.K., Dwarakanath B.S. and Jain V. (1996). Improving cancer radiotherapy with 2-deoxy-D-glucose: phase I/II clinical trials on human cerebral gliomas. Int. J. Radiat. Oncol. Biol. Phys. 35, 103-111.

Moreno-Sanchez R., Rodriguez-Enriquez S., Marin-Hernandez A. and Saavedra E. (2007). Energy metabolism in tumor cells. FEBS J. 274, 1393-1418.

Pedersen P.L. (1978). Tumor mitochondria and the bioenergetics of cancer cells. Prog. Exp. Tumor Res. 22, 190-274.

Pollard P.J., Briere J.J., Alam N.A., Barwell J., Barclay E., Wortham N.C., Hunt T., Mitchell M., Olpin S., Moat S.J., Hargreaves I.P., Heales S.J., Chung Y.L., Griffiths J.R., Dalgleish A., McGrath J.A., Gleeson M.J., Hodgson S.V., Poulsom R., Rustin P. and Tomlinson I.P. (2005). Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum. Mol. Genet. 14, 2231-2239.

Pollard P.J., Wortham N.C. and Tomlinson I.P. (2003). The TCA cycle and tumorigenesis: the examples of fumarate hydratase and succinate dehydrogenase. Ann. Med. 35, 632-639.

Stackhouse B.L., Williams H., Berry P., Russell G., Thompson P., Winter J.L. and Kute T. (2005). Measurement of glut-1 expression using tissue microarrays to determine a race specific prognostic marker for breast cancer. Breast Cancer Res. Treat. 93, 247-253.

Sun F., Huo X., Zhai Y., Wang A., Xu J., Su D., Bartlam M. and Rao Z. (2005). Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121, 1043-1057.

Tavassoli F.A., Devilee P., International Agency for Research on Cancer and World Health Organization (2003). Pathology and genetics of tumours of the breast and female genital organs. IAPS Press. Lyon.

van Nederveen F.H., Gaal J., Favier J., Korpershoek E., Oldenburg R.A., de Bruyn E.M., Sleddens H.F., Derkx P., Riviere J., Dannenberg H., Petri B.J., Komminoth P., Pacak K., Hop W.C., Pollard P.J., Mannelli M., Bayley J.P., Perren A., Niemann S., Verhofstad A.A., de Bruine A.P., Maher E.R., Tissier F., Meatchi T., Badoual C., Bertherat J., Amar L., Alataki D., Van Marck E., Ferrau F., Francois J., de Herder W.W., Peeters M.P., van Linge A., Lenders J.W., Gimenez-Roqueplo A.P., de Krijger R.R. and Dinjens W.N. (2009). 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. 10, 764-771.

Vullo D., Franchi M., Gallori E., Pastorek J., Scozzafava A., Pastorekova S. and Supuran C.T. (2003). Carbonic anhydrase inhibitors: inhibition of the tumor-associated isozyme IX with aromatic and heterocyclic sulfonamides. Bioorg. Med. Chem. Lett. 13, 1005-1009.

Warburg O. (1956). On the origin of cancer cells. Science 123, 309-314.

Yeo E.J., Chun Y.S., Cho Y.S., Kim J., Lee J.C., Kim M.S. and Park J.W. (2003). YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. J. Natl. Cancer Inst. 95, 516-525.

Younes M., Brown R.W., Mody D.R., Fernandez L. and Laucirica R. (1995). GLUT1 expression in human breast carcinoma: correlation with known prognostic markers. Anticancer Res. 15, 2895-2898.

Younes M., Brown R.W., Stephenson M., Gondo M. and Cagle P.T. (1997). Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer 80, 1046-1051.

Accepted May 7, 2014