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Original Article

Expression of sarcosine-metabolizing enzymes in thyroid cancer

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Abstract: The purpose of this study was to investigate the expression of sarcosine metabolism-related proteins according to thyroid cancer subtypes and the implications of these findings. We performed tissue microarrays of 557 thyroid cancer cases (papillary thyroid carcinoma [PTC]: 344, follicular carcinoma [FC]: 112, medullary carcinoma [MC]: 70, poorly differentiated carcinoma [PDC]: 23, and anaplastic carcinoma [AC]: 8) and 152 follicular adenoma cases. Immunohistochemical staining for sarcosine metabolism-related molecules [glycine *N*-methyltransferase (GNMT), sarcosine dehydrogenase (SARDH), and l-pipecolic acid oxidase (PIPOX)] was conducted, and the results were analyzed based on clinicopathologic parameters. Results: The expression of SARDH and PIPOX was different depending on thyroid cancer subtypes. PTC showed higher expression than other subtypes (P<0.001). Among PTC, follicular variant (FV) showed lower expression than conventional type (P = 0.010 and P<0.001) and PTC with BRAF V600E mutation showed higher expression than PTC without BRAF V600E mutation (P<0.001). In univariate analysis, PIPOX positivity was associated with shorter overall survival (OS) in PTC (P = 0.024). In conclusion, the expression of sarcosine metabolism-related proteins varied according to thyroid cancer subtypes. SARDH and PIPOX showed higher expression in PTC; among PTC, FVPTC showed lower expression and PTC with BRAF V600E mutation showed higher expression.

Keywords: Metabolism, sarcosine, thyroid cancer

Introduction

Sarcosine (N-methylglycine) is a non-proteinogenic amino acid created during glycine synthesis and degradation. The major enzymes involved in the sarcosine metabolism pathway are glycine *N*-methyltransferase (GNMT), sarcosine dehydrogenase (SARDH), and l-pipecolic acid oxidase (PIPOX). Sarcosine is formed by transferring the methyl group from S-adenosylmethionine to glycine via the enzyme GNMT. Sarcosine-metabolizing enzymes SARDH and PIPOX make glycine via oxidative demethylation of sarcosine [1]. Sarcosine has been reported to be a potential oncometabolite [2, 3]. In particular, sarcosine in prostate cancer was shown to be a sensitive tumor biomarker and to be associated with tumor progression and metastasis [2, 3].

Thyroid cancer is a common malignancy, accounting for about 1% of the total population. The common subtypes are papillary thyroid carcinoma (PTC), follicular carcinoma (FC), medullary carcinoma (MC), poorly differentiated carcinoma (PDC), and anaplastic carcinoma (AC). Cell origin, clinical manifestation, metastatic pattern, and clinical prognosis vary according to subtypes [4]. The expression of sarcosine-metabolizing enzymes was reported in other endocrine-related cancers such as prostate cancer and breast cancer [5, 6], and it was found to be associated with tumor progression and metastasis [5]. Previous studies revealed that thyroid hormone is related to regulation of the enzymes involved in methyl group metabolism such as GNMT [7]. The presence of sarcosine-metabolizing enzymes in thyroid cancer is thus highly likely. However, a direct investigation has not been previously performed. The purpose of this study was thus to investigate the expression of sarcosine metabolism-related proteins according to thyroid cancer subtypes and to elucidate potential implications.

Materials and methods

Patient selection

For PTC, we included patients who underwent surgery after the diagnosis of PTC at Severance

Table 1. Source, clone, and dilution of antibodies used in this study

Antibody	Company	Clone	Dilution
Sarcosine metabolism-related proteins			
GNMT	Abcam, Cambridge, UK	Polyclonal	1:100
SARDH	Abcam, Cambridge, UK	Polyclonal	1:100
PIPOX	Abcam, Cambridge, UK	Polyclonal	1:100

GNMT, glycine *N*-methyltransferase; SARDH, sarcosine dehydrogenase; PIPOX, l-pipecolic acid oxidase.

Hospital between January 2012 and December 2013. For other subtypes, we included patients who underwent surgery and were diagnosed at Severance Hospital between January 2000 and December 2014. Patients treated with neoadjuvant chemotherapy were excluded. This study was approved by the Institutional Review Board of Yonsei University Severance Hospital. All cases were retrospectively reviewed by a thyroid pathologist (Koo JS), and histologic review was performed on Hematoxylin-Eosin (H&E)-stained slides. Clinicopathologic data were obtained from patient medical records and included age at diagnosis, disease recurrence, metastasis, current status, and length of follow-up. Tumor size, location (right or left lobe), extent (confined to the thyroid parenchyma or with extrathyroidal spread) and number of metastatic lymph nodes were also recorded from slide review and surgical pathology reports.

Tissue microarray

Representative areas were selected on H&E-stained slides, and a corresponding spot was marked on the surface of the matching paraffin block. Five-millimeter core biopsies were taken from selected areas and placed into a 5×4-mm recipient block. More than two tissue cores were extracted from each case to minimize extraction bias. Each tissue core was assigned a unique tissue microarray location number that was linked to a database containing other clinicopathologic data.

Immunohistochemistry

Antibodies used for immunohistochemistry are listed in **Table 1**. All immunohistochemistry was performed with formalin-fixed, paraffin-embedded tissue sections using an automatic immunohistochemistry staining device (Benchmark XT, Ventana Medical Systems, Tucson, AZ, USA). Briefly, 5-μm-thick formaldehyde fixed paraffin-embedded tissue sections were transferred onto adhesive slides and dried at 62°C for 30 minutes. Standard heat epitope retrieval was performed for 30 minutes in ethylene diamine tetraacetic acid, pH 8.0, in the autostainer. The samples were then incubated with primary antibodies. After incubation with primary antibodies, the sections were subsequently incubated with biotinylated anti-mouse immunoglobulins, peroxidase-labeled streptavidin (LSAB kit, DakoCytomation, Glostrup, Denmark), and 3, 30-diaminobenzidine. Negative control samples were processed without the primary antibody. Slides were counterstained with Harris hematoxylin. Positive control tissue was used per the manufacturer's recommendations.

Interpretation of immunohistochemical staining

Immunohistochemical markers were detected by light microscopy. The stained slides were evaluated semi-quantitatively using a previously reported method [8]. Tumor cell staining was assessed as 0: negative or weak immunostaining in <1% of the tumor, 1: focal expression in 1-10% of tumor, 2: positive in 11-50% of the tumor, and 3: positive in 51-100% of the tumor. The entire tumor area was evaluated, and a score of 2 or more were defined as positive.

Statistical analysis

Data were analyzed using SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA). To determine statistical significance, Student's *t* and Fisher's exact tests were used for continuous and categorical variables, respectively. To analyze data with multiple comparisons, a corrected *p*-value with the application of Bonferroni multiple comparison procedure was used. Statistical significance was set at $P<0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor

Table 2. Expression of sarcosine metabolism-related proteins according to thyroid cancer subtypes

Parameters	Total N = 557 (%)	PTC N = 344 (%)	FC N = 112 (%)	MC N = 70 (%)	PDC N = 23 (%)	AC N = 8 (%)	*P*-value
GNMT							n/a
Negative	557 (100.0)	344 (100.0)	112 (100.0)	70 (100.0)	23 (100.0)	8 (100.0)	
Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
SARDH							<0.001
Negative	443 (79.5)	240 (69.8)	103 (92.0)	70 (100.0)	22 (95.7)	8 (100.0)	
Positive	114 (20.5)	104 (30.2)	9 (8.0)	0 (0.0)	1 (4.3)	0 (0.0)	
PIPOX							<0.001
Negative	296 (53.2)	124 (36.2)	101 (90.2)	43 (61.4)	20 (87.0)	8 (100.0)	
Positive	260 (46.8)	219 (63.8)	11 (9.8)	27 (38.6)	3 (13.0)	0 (0.0)	

PTC, papillary thyroid carcinoma; FC, follicular carcinoma; MC, medullary carcinoma; PDC, poorly differentiated carcinoma; AC, anaplastic carcinoma; GNMT, glycine *N*-methyltransferase; SARDH, sarcosine dehydrogenase; PIPOX, l-pipecolic acid oxidase.

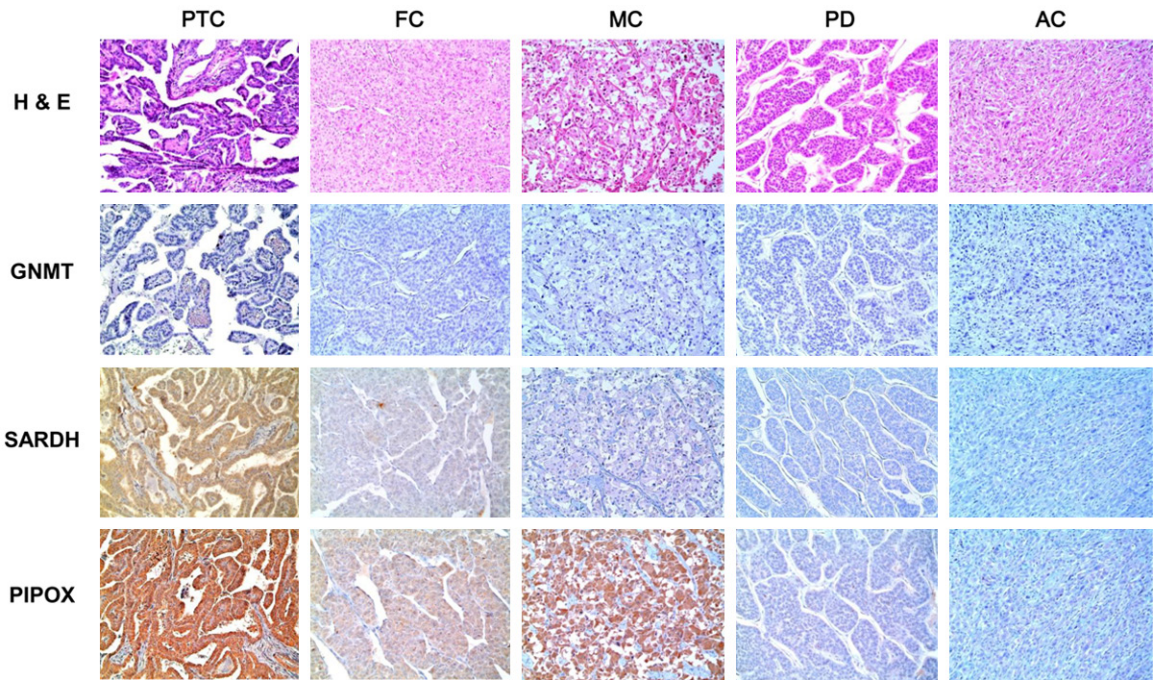

Figure 1. Expression of sarcosine metabolism-related proteins in thyroid cancer. Glycine *N*-methyltransferase (GNMT) was negative in all thyroid cancers. The expression of sarcosine dehydrogenase (SARDH) and pipecolic acid oxidase (PIPOX) was higher in PTC than in other subtypes.

recurrence and overall survival (OS). Multivariate regression analysis was performed using the Cox proportional hazards model.

Results

Basal characteristics of thyroid cancer

This study included 557 cases of thyroid cancer: 344 cases of PTC, 112 cases of FC, 70 cases of MC, 23 cases of PDC, and eight cases of AC. The basal characteristics of PTC are listed in Supplementary Table 1; it was composed of 304 cases of conventional type and 40 cases of FVPTC. In FVPTC, the proportion of tumors with expanding margins (P = 0.002) was higher. In addition, 238 cases of PTC (69.2%) were found to have BRAF V600E mutation. The proportion of infiltrative tumor margin was higher (P = 0.004) in PTC with BRAF V600E mutation and lower in that of FVPTC (P<0.001). FC was composed of 99 cases of minimally inva-

Table 3. Expression of sarcosine metabolism-related proteins according to PTC histologic subtypes and BRAF V600E mutation status

Parameters	Total N = 344 (%)	Histologic subtype		*P*-value	BRAF V600E mutation status		*p*-value
		Follicular variant N = 40 (%)	Conventional type N = 304 (%)		No mutation N = 106 (%)	Mutation N = 238 (%)	
GNMT				n/a			n/a
Negative	344 (100.0)	40 (100.0)	304 (100.0)		106 (100.0)	238 (100.0)	
Positive	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
SARDH				0.010			<0.001
Negative	240 (69.8)	35 (87.5)	205 (67.4)		101 (95.3)	139 (58.4)	
Positive	104 (30.2)	5 (12.5)	99 (32.6)		5 (4.7)	99 (41.6)	
PIPOX				<0.001			<0.001
Negative	125 (36.3)	26 (65.0)	99 (32.6)		72 (67.9)	53 (22.3)	
Positive	219 (63.7)	14 (35.0)	205 (67.4)		34 (32.1)	185 (77.7)	

PTC, papillary thyroid carcinoma; GNMT, glycine *N*-methyltransferase; SARDH, sarcosine dehydrogenase; PIPOX, l-pipecolic acid oxidase.

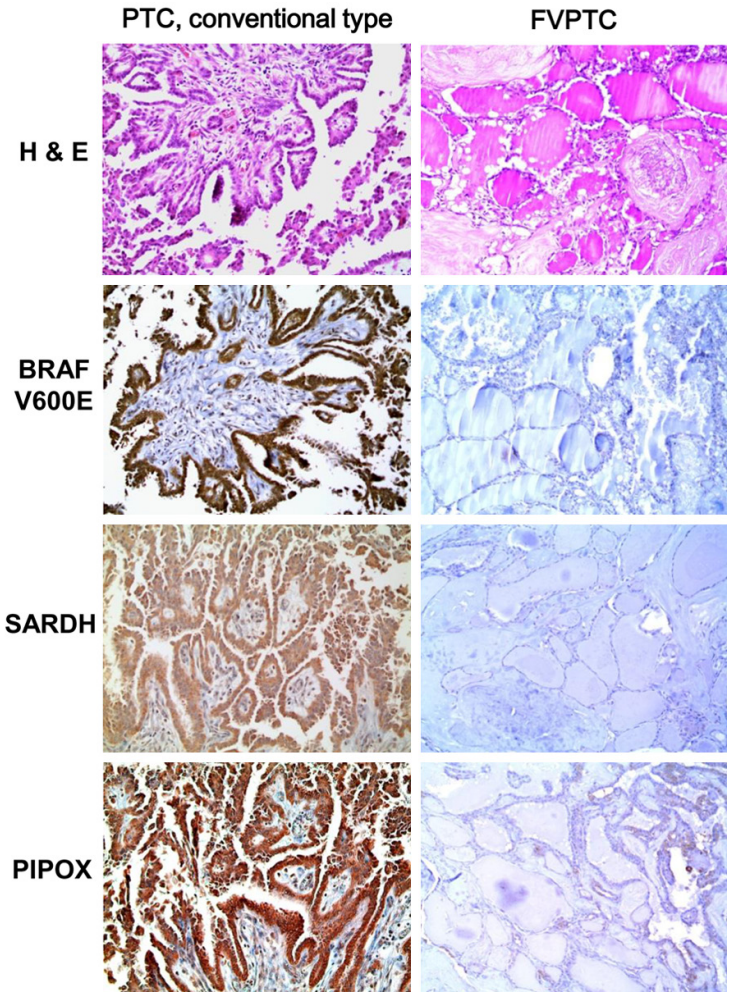

Figure 2. Expression of sarcosine metabolism-related proteins in PTC. SARDH and PIPOX expression was lower in FVPTC and PTC without BRAF V600E mutation.

sive type and 13 cases of widely invasive type. In the case of widely invasive type, the proportions of larger tumors (>2.0 cm) (P = 0.040), vascular invasion (P = 0.028), extrathyroidal involvement (P<0.001), and distant metastasis (P = 0.003) were higher than minimally invasive type (Supplementary Table 2). The basal characteristics of MC, PDC, and AC are described in Supplementary Table 3.

Expression of sarcosine metabolism-related proteins in thyroid cancer

We investigated the expression of sarcosine metabolism-related proteins in thyroid cancer. GNMT was negative in all thyroid cancer. The expression of SARDH and PIPOX varied according to thyroid cancer subtypes and was higher in PTC than in other subtypes (P<0.001, **Table 2** and **Figure 1**).

Expression of sarcosine metabolism-related proteins according to thyroid cancer subtypes

We investigated the expression of sarcosine metabolism-related proteins by thyroid cancer subtypes. In PTC, SARDH and PIPOX expression varied depending on histologic subtypes and BRAF V600E mutation status. FVPTC showed lower expression than conventional type (P = 0.010, and P<0.001), and PTC with BRAF V600E mutation showed

Table 4. Expression of sarcosine metabolism-related proteins in follicular neoplasms

Parameters	Total N = 264 (%)	Follicular adenoma N = 152 (%)	Follicular carcinoma N = 112 (%)	*P*-value
GNMT				n/a
Negative	264 (100.0)	152 (100.0)	112 (100.0)	
Positive	0 (0.0)	0 (0.0)	0 (0.0)	
SARDH				0.184
Negative	249 (94.3)	146 (96.1)	103 (92.0)	
Positive	15 (5.7)	6 (3.9)	9 (8.0)	
PIPOX				0.237
Negative	244 (92.4)	143 (94.1)	101 (90.2)	
Positive	20 (7.6)	9 (5.9)	11 (9.8)	

GNMT, glycine *N*-methyltransferase; SARDH, sarcosine dehydrogenase; PIPOX, l-pipecolic acid oxidase.

Table 5. Expression of sarcosine metabolism-related proteins according to FC histologic subtypes

Parameters	Total N = 112 (%)	FC, widely invasive type N = 13 (%)	FC, minimally invasive type N = 99 (%)	*P*-value
GNMT				n/a
Negative	112 (100.0)	13 (100.0)	99 (100.0)	
Positive	0 (0.0)	0 (0.0)	0 (0.0)	
SARDH				0.595
Negative	103 (92.0)	13 (100.0)	90 (90.9)	
Positive	9 (8.0)	0 (0.0)	9 (9.1)	
PIPOX				0.614
Negative	101 (90.2)	11 (84.6)	90 (90.9)	
Positive	11 (9.8)	2 (15.4)	9 (9.1)	

FC, follicular carcinoma; GNMT, glycine *N*-methyltransferase; SARDH, sarcosine dehydrogenase; PIPOX, l-pipecolic acid oxidase.

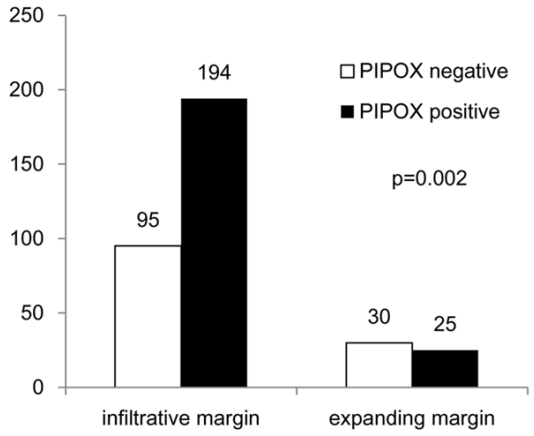

Figure 3. Correlation between clinicopathologic factors and sarcosine metabolism-related proteins in PTC.

higher expression than PTC without BRAF V600E mutation (P<0.001, **Table 3** and **Figure 2**).

There were no differences in the expression of sarcosine metabolism-related proteins between follicular adenoma and FC (**Table 4**). Also, there was no difference between minimally invasive and widely invasive FC (**Table 5**).

When the relationship between clinicopathologic factors and the expression of sarcosine metabolism-related proteins was reviewed in thyroid cancer, PIPOX positivity was associated with infiltrative margins in PTC (P = 0.002, **Figure 3**).

Expression of sarcosine metabolism-related proteins and patient prognosis in thyroid cancer

We investigated the relationship between expression of sarcosine metabolism-related proteins and patient prognosis in thyroid cancer. In univariate analysis, PIPOX positivity was associated with shorter OS in PTC (P = 0.024, **Table 6** and **Figure 4**). In multivariate Cox analysis, lymph node metastasis was independently associated with shorter disease-free survival (hazard ratio: 6.641, 95% CI: 1.459-30.24, P = 0.014) and age ≥45 years was associated with

Table 6. Univariate analysis of sarcosine metabolism-related protein expression in PTC and disease-free/overall survival using the log-rank test

Parameter	Number of patients/recurrence/death	Disease-free survival		Overall survival	
		Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value
GNMT			n/a		n/a
Negative	344/18/18	n/a		n/a	
Positive	0/0/0	n/a		n/a	
SARDH			0.871		0.118
Negative	240/13/10	106 (103-108)		108 (106-110)	
Positive	104/5/8	107 (103-111)		105 (100-109)	
PIPOX			0.488		0.024
Negative	125/8/2	106 (102-110)		111 (109-112)	
Positive	219/10/16	107 (105-110)		106 (103-109)	

PTC, papillary thyroid carcinoma; GNMT, glycine *N*-methyltransferase; SARDH, sarcosine dehydrogenase; PIPOX, l-pipecolic acid oxidase.

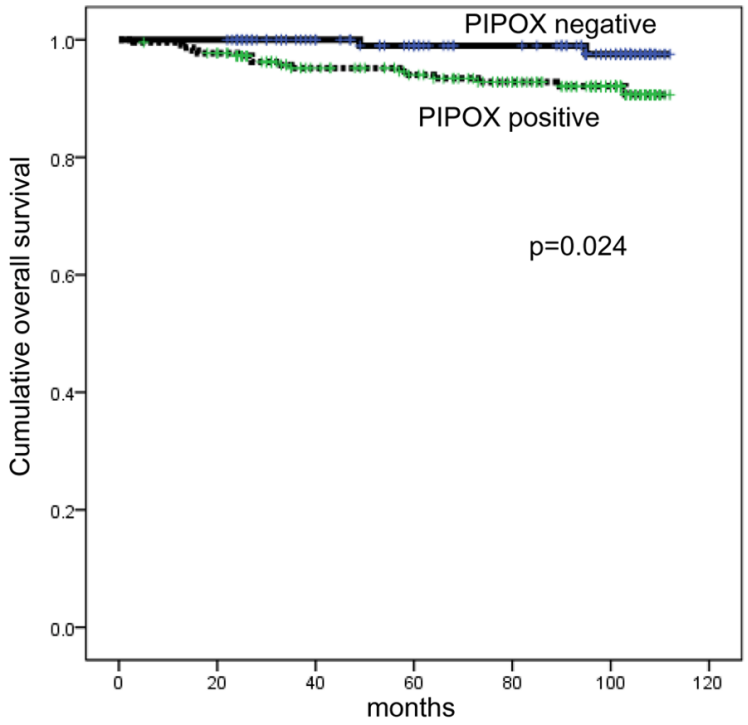

Figure 4. Expression of sarcosine metabolism-related proteins and patient prognosis in PTC.

shorter OS (hazard ratio: 14.42, 95% CI: 1.889-110.1, P = 0.010) (**Table 7**).

Discussion

In this study, the expression of sarcosine metabolism-related proteins varied by thyroid cancer subtypes. SARDH and PIPOX were more highly expressed in PTC than in other subtypes. Direct comparison with other research is not possible because there are few other studies on sarcosine metabolism in thyroid cancer. However, similar differences in PIPOX expression between invasive ductal carcinoma and invasive lobular carcinoma of breast cancer have been reported [9]. Although tumors may originate from the same cell type, different expression can be seen depending on the histologic subtypes. In fact, in thyroid cancer, the expression of SARDH and PIPOX was lower in FVPTC than in conventional PTC. SARDH and PIPOX expression was highest in conventional PTC followed by FVPTC and FC; therefore, SARDH and PIPOX were proven to show lower expression and more follicular differentiation. Further investigation regarding the biologic significance of these findings will be required. This study also showed that PTC with BRAF V600E mutation was associated with higher expression of SARDH and PIPOX. In PTC with BRAF V600E mutation, the expression of glycolysis related proteins was reported to be higher in another study [10]. Depending on whether the BRAF V600E mutation is present in PTC, the metabolic characteristics may vary [10]. This study also confirms that sarcosine metabolism-relat-

Table 7. Multivariate analysis of PTC survival

Included parameters	Disease-free survival			Overall survival		
	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
Age (years)			0.620			0.010
<45 versus ≥45	1.276	0.488-3.338		14.42	1.889-110.1	
Tumor size (cm)			0.828			0.133
≤2.0 versus >2.0	1.124	0.392-3.222		2.161	0.792-5.901	
Tumor extension			0.278			0.466
Intrathyroidal versus extrathyroidal	0.583	0.220-1.544		0.675	0.235-1.942	
Histologic type			0.634			n/a
Follicular versus conventional	1.449	0.315-6.658		n/a	n/a	
Lymph node metastasis			0.014			0.092
No versus yes	6.641	1.459-30.24		2.626	0.854-8.069	
BRAF V600E mutation			0.381			n/a
No versus yes	0.612	0.204-1.839		n/a	n/a	
PIPOX			0.601			0.301
Negative versus positive	0.757	0.267-2.149		2.182	0.198-9.555	

PTC, papillary thyroid carcinoma; PIPOX, l-pipecolic acid oxidase.

ed proteins are more highly expressed in PTC with BRAF V600E mutation. The possible mechanism was raised why PTC with BRAF V600E mutation showing high metabolic activity. It is that BRAF mutation activates mitogen-activated protein kinase downstream targets such as c-myc and HIF-1a and thereby increases glucose metabolism [11, 12]. Further studies on the association of sarcosine metabolism-related proteins and PTC with BRAF V600E mutation seem to be necessary.

In this study, the expression of SARDH was not shown in MC; in contrast, the expression of PIPOX had a large percentage in MC next to PTC (38.6%). The mechanism by which PIPOX is expressed in MC is unknown. However, the association between PIPOX and HER-2 can be considered a possible cause. Because it has been previously reported that the expression of HER-2 is higher in MC [13] and the expression of PIPOX has been found to be associated with HER-2 positivity in breast cancer [6, 14], further studies on the relationship between HER-2 and PIPOX in MC are needed.

In this study, the expression of PIPOX was found to be associated with poor prognosis in PTC. The expression of PIPOX in metastatic breast cancer was also associated with poor prognosis, which is compatible with the results of this study [15]. In contrast, PIPOX negativity was reported to be associated with a poor prognosis in primary breast cancer [6]. The mechanism and utility of PIPOX as a prognostic marker thus warrants further investigation.

In conclusion, the expression of sarcosine metabolism-related proteins varied according to thyroid cancer subtypes. SARDH and PIPOX were highly expressed in PTC. Among the PTC subtypes, there was lower expression of SARDH and PIPOX in FVPTC and higher expression in PTC with BRAF V600E mutation.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Basal characteristics of PTC

Parameters	Total N = 344 (%)	Histologic subtype		*P*-value	BRAF V600E mutation status		*P*-value
		Conventional type N = 304 (%)	Follicular variant N = 40 (%)		No mutation N = 106 (%)	Mutation N = 238 (%)	
Age (years)				0.741			0.089
<45	155 (45.1)	136 (44.7)	19 (47.5)		55 (51.9)	100 (42.0)	
≥45	189 (54.9)	168 (55.3)	21 (52.5)		51 (48.1)	138 (58.0)	
Sex				0.969			0.235
Male	68 (19.8)	60 (19.7)	8 (20.0)		25 (23.6)	43 (18.1)	
Female	276 (80.2)	244 (80.3)	32 (80.0)		81 (76.4)	195 (81.9)	
Tumor size (cm)				0.951			0.506
≤2.0	272 (79.1)	240 (78.9)	32 (80.0)		80 (75.5)	192 (80.7)	
>2.0, ≤4.0	65 (18.9)	58 (19.1)	7 (17.5)		23 (21.7)	42 (17.6)	
>4.0	7 (2.0)	6 (2.0)	1 (2.5)		3 (2.8)	4 (1.7)	
Tumor margin				0.002			0.004
Infiltrative	289 (84.0)	262 (67.5)	27 (67.5)		80 (75.5)	209 (87.8)	
Expanding	55 (16.0)	42 (13.8)	13 (32.5)		26 (24.5)	29 (12.2)	
Tumor extension				0.330			0.177
Intrathyroidal	106 (30.8)	91 (29.9)	15 (37.5)		38 (35.8)	68 (28.6)	
Extrathyroidal	238 (69.2)	213 (70.1)	25 (62.5)		68 (64.2)	170 (71.4)	
Histologic subtype							<0.001
Conventional					81 (76.4)	223 (93.7)	
Follicular					25 (23.6)	15 (6.3)	
LN metastasis				0.175			0.075
No	138 (40.1)	118 (38.8)	20 (50.0)		50 (47.2)	88 (37.0)	
Yes	206 (59.9)	186 (61.2)	20 (50.0)		56 (52.8)	150 (63.0)	
Distant metastasis				0.944			0.446
No	326 (94.8)	288 (94.7)	38 (95.0)		99 (93.4)	227 (95.4)	
Yes	18 (5.2)	16 (5.3)	2 (5.0)		7 (6.6)	11 (4.6)	

PTC, papillary thyroid carcinoma.

Supplementary Table 2. Basal characteristics of FC

Parameters	Total N = 112 (%)	FC, minimally invasive type N = 99 (%)	FC, widely invasive type N = 13 (%)	*P*-value
Age (years)				0.255
<45	51 (45.5)	47 (47.5)	4 (30.8)	
≥45	61 (54.5)	52 (52.5)	9 (69.2)	
Sex				0.233
Male	28 (25.0)	23 (23.2)	5 (38.5)	
Female	84 (75.0)	76 (76.8)	8 (61.5)	
Tumor size (cm)				0.040
≤2.0	34 (30.4)	34 (34.3)	0 (0.0)	
>2.0, ≤4.0	49 (43.8)	41 (41.4)	8 (61.5)	
>4.0	29 (25.9)	24 (24.2)	5 (38.5)	
Capsular invasion				0.147
No	14 (12.5)	14 (14.1)	0 (0.0)	
Yes	98 (87.5)	85 (85.9)	13 (100.0)	
Vascular invasion				0.028
No	66 (58.9)	62 (62.6)	4 (30.8)	
Yes	46 (41.1)	37 (37.4)	9 (69.2)	
Tumor extension				<0.001
Intrathyroidal	95 (84.8)	89 (89.9)	6 (46.2)	
Extrathyroidal	17 (15.2)	10 (10.1)	7 (53.8)	
LN metastasis				0.220
No	110 (98.2)	98 (99.0)	12 (92.3)	
Yes	2 (1.8)	1 (1.0)	1 (7.7)	
Distant metastasis				0.003
No	101 (90.2)	93 (93.9)	8 (61.5)	
Yes	11 (9.8)	6 (6.1)	5 (38.5)	

FC: follicular carcinoma.

Supplementary Table 3. Basal characteristics of MC, PDC, and AC

Parameters	MC, n = 70 (%)	PDC, n = 23 (%)	AC, n = 8 (%)
Age (years)			
<45	21 (30.0)	4 (17.4)	0 (0.0)
≥45	49 (70.0)	19 (82.6)	8 (100.0)
Sex			
Male	22 (31.4)	10 (43.5)	1 (12.5)
Female	48 (68.6)	13 (56.5)	7 (87.5)
Tumor size (cm)			
≤2.0	53 (75.7)	8 (34.8)	0 (0.0)
>2.0, ≤4.0	14 (20.0)	9 (39.1)	1 (12.5)
>4.0	3 (4.3)	6 (26.1)	7 (87.5)
Tumor margin			
Infiltrative	45 (64.3)	17 (73.9)	8 (100.0)
Expanding	25 (35.7)	6 (26.1)	0 (0.0)
Tumor extension			
Intrathyroidal	52 (74.3)	11 (47.8)	0 (0.0)
Extrathyroidal	18 (25.7)	12 (52.2)	8 (100.0)
LN metastasis			
No	47 (67.1)	22 (95.7)	4 (50.0)
Yes	23 (32.9)	1 (4.3)	4 (50.0)
Distant metastasis			
No	67 (95.7)	16 (69.6)	8 (100.0)
Yes	3 (4.3)	7 (30.4)	0 (0.0)

MC, medullary carcinoma; PDC, poorly differentiated carcinoma; AC, anaplastic carcinoma.