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Original Article

Differential expression of serine and glycine metabolism-related proteins between follicular neoplasm and Hürthle cell neoplasm

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Abstract: The aim of the study was to investigate the expression of serine/glycine-related proteins in Hürthle cell neoplasm (HCN) and follicular neoplasm (FN) and to explore its associated implications. Tissue microarrays were constructed with 265 cases of FN (follicular carcinoma [FC]: 112 and follicular adenoma [FA]: 153) and 107 cases of HCN (Hürthle cell carcinoma [HCC]: 27 and Hürthle cell adenoma [HCA]: 80). The serine/glycine-related proteins PHGDH, PSAT1, SHMT1, and GLDC were evaluated using immunohistochemical staining. The expression of SHMT1 and PHGDH was higher in HCN compared to FN (P<0.001 and P=0.048). SHMT1 expression was highest in HCC, followed by HCA, FA, and FC (P<0.001), and PHGDH expression was highest in HCA, followed by HCC, FC, and FA (P=0.041). In FC, SHMT1 negativity was associated with extrathyroidal extension (P=0.019). In univariate analysis, PSAT1 negativity was associated with shorter overall survival (P<0.001). The expression of serine/glycine-related proteins differed between FN and HCN. The expression of both SHMT1 and PHGDH was higher in HCN compared to FN. The clinical implications of this study are that the serine/glycine metabolism pathway could be a possible therapeutic target in HCC.

Keywords: Metabolism, Hürthle cell, thyroid, serine, glycine

Introduction

A common metabolic trait of malignant tumors is explained by the Warburg effect, with a metabolic shift from oxidative phosphorylation (OXPHOS) to glycolysis within the mitochondria of tumor cells [1]. As a consequence of this altered metabolism, the glycolytic intermediates increase in tumor cells, where active glycolysis occurs. Recently, it was reported that the metabolism of glycolytic intermediates in tumor cells is involved in tumorigenesis. The glycine and serine metabolic pathways are representative of these metabolic pathways [2-5]. In the serine biosynthesis pathway, 3-phosphoglycerate (3PG) formed during glycolysis is oxidized to 3-phosphohydroxypyruvate (pPYR) by phosphoglycerate dehydrogenase (PHGDH), and pPYR is transaminated to phosphoserine (pSER) by phosphoserine aminotransferase (PSAT). Next, pSER is dephosphorylated by phosphoserine phosphatase (PSPH) as serine. Similarly, in glycine metabolism, methylene-tetrahydrofolate is generated by glycine decarboxylase (GLDC) from glycine. Serine and glycine metabolism can be linked by serine hydroxymethyltransferase (SHMT), which causes reversible conversion of serine and glycine [6].

Hürthle cell neoplasm (HCN) of the thyroid gland is a variant of follicular neoplasm (FN). Hürthle cell adenoma (HCA) accounts for 10-15% of follicular adenoma (FA), and Hürthle cell carcinoma (HCC) accounts for 20-25% of follicular carcinoma (FC) [7]. Hürthle cells originate from follicular epithelial cells and are characterized by abundant granular cytoplasm and nuclei with prominent nucleoli. According to the World Health Organization (WHO) classification of endocrine organs, HCN is regarded as a variant of FN [8]. However, some evidence suggests that HCN should be classified as a distinct disease entity. First, compared to FC, HCC can metastasize to lymph nodes [9, 10], has higher rates of recurrence, and is associated with higher disease-related mortality [10-13].

Second, a genomic dissection study of HCC revealed that HCC has distinct mutational, transcriptional, and copy number profiles from papillary thyroid cancer or follicular thyroid cancer [14]. Among molecular features, the TERT C228T promoter mutation is reported to be common in HCN [15]. In addition, previous studies have reported different FDG-PET characteristics between HCA and FA; higher focal FDG uptake and higher SUVmax were noted in HCA compared to FA [16]. Collectively, these findings suggest that there are differences in metabolic characteristics and tumor biology between HCN and FN. Therefore, in this study, we investigated the expression of serine/glycine-related proteins in HCN and FN and discuss the associated implications.

Materials and methods

Patient selection

Patients diagnosed with FN and HCN after surgery at Severance Hospital from January 2000 to December 2012 were included. Patients who underwent preoperative therapy 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 histological evaluation was conducted by hematoxylin and 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 parenchyme or with extrathyroidal spread), and number of metastatic lymph nodes were also noted from review of the slides and surgical pathology reports.

Tissue microarray

Representative areas were selected on hematoxylin-eosin-stained slides, and a corresponding spot was marked on the surface of the matching paraffin block. Five-mm core biopsies were collected from selected areas and placed into a 5×4 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 System, 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 an autostainer. The samples were then incubated with primary antibodies, followed by incubation with biotinylated anti-mouse immunoglobulins, peroxidase-labeled streptavidin (LSAB kit, DakoCytomation), and 3,30-diaminobenzidine. Slides were counterstained with Harris hematoxylin. Negative control samples were processed without the primary antibody. Positive control tissue was used as per the manufacturer's recommendations. Optimal primary antibody incubation times and concentrations were determined by serial dilution for each immunohistochemical assay using a tissue block fixed and embedded as in the experiments.

Interpretation of immunohistochemical staining

Immunohistochemical markers were assessed by light microscopy and semi-quantitatively evaluated, as previously described [17]. Tumor cell staining was scored as 0: negative or weak immunostaining in <1% of the tumor/stroma, 1: focal expression in 1-10% of the tumor, 2: positive in 11%-50% of the tumor, and 3: positive in 51%-100% of the tumor. These evaluations were applied over the entire area of the tumor, which was scored as follows: score 0: negative and score 1: positive for PSAT1 and score 0-1: negative and score 2-3: positive for PHGDH, SHMT1, and GLDC.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp. Released 2012. Armonk, NY, USA). Student's t and Fisher's exact tests were used for continuous and categorical variables, respectively. In the case of analyzing data with multiple comparisons, a corrected *p*-value with the application

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

Antibody	Clone	Dilution	Company
PHGDH	Polyclonal	1:100	Abcam, Cambridge, UK
PSAT1	Polyclonal	1:100	Abcam, Cambridge, UK
SHMT1	Polyclonal	1:100	Abcam, Cambridge, UK
GLDC	Polyclonal	1:100	Abcam, Cambridge, UK

PHGDH, phosphoglycerate dehydrogenase, PSAT1, phosphoserine aminotransferase-1, SHMT1, serine hydroxymethyltransferase-1, GLDC, glycine decarboxylase.

Table 2. Expression of serine and glycine metabolism-related proteins in follicular neoplasm and Hürthle cell neoplasm

Parameters	Total N=372 (%)	Follicular neoplasm n=265 (%)	Hürthle cell neoplasm n=107 (%)	*p*-value
PSAT1				0.165
Negative	300 (80.6%)	219 (82.6%)	81 (75.7%)	
Positive	72 (19.4%)	46 (17.4%)	26 (24.3%)	
GLDC				0.458
Negative	332 (89.2%)	234 (88.3%)	98 (91.6%)	
Positive	40 (10.8%)	31 (11.7%)	9 (8.4%)	
SHMT1				<0.001
Negative	58 (15.6%)	56 (21.1%)	2 (1.9%)	
Positive	314 (84.4%)	209 (78.9%)	105 (98.1%)	
PHGDH*				0.048
Negative	192 (51.6%)	147 (55.5%)	45 (42.1%)	
Positive	179 (48.1%)	117 (44.2%)	62 (57.9%)	

PSAT1, phosphoserine aminotransferase-1; GLDC, glycine decarboxylase; SHMT1, serine hydroxymethyltransferase-1; PHGDH, phosphoglycerate dehydrogenase. *Immunohistochemistry was not carried out in one case.

of the Bonferroni multiple comparison procedure was used. Statistical significance was set to P<0.05. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence and overall survival. Multivariate regression analysis was performed using the Cox proportional hazards model.

Results

Basal characteristics of follicular neoplasm and Hürthle cell neoplasm

A total of 265 FN were included in this study: 153 cases of FA and 112 cases of FC. Of the FC cases, 99 were minimally invasive type and 13 were widely invasive type. The clinicopathologic features of FC patients are presented in Supplementary Table 1. For HCN, a total of 107 cases were examined: 80 cases of HCA and 27 cases of HCC. The clinicopathologic features of HCN patients are presented in Supplementary Table 2.

Expression of serine/glycine-related proteins in follicular neoplasm and Hürthle cell neoplasm

We investigated the expression of serine/glycine-related proteins in FN and HCN. The expression of SHMT1 and PHGDH was higher in HCN compared to FN (P<0.001 and P=0.048 respectively, **Table 2** and **Figure 1**). Differences were noted in the expression of serine/glycine-related proteins SHMT1 and PHGDH between FA, FC, HCA, and HCC. SHMT1 expression was highest in HCC, followed by HCA, FA, and FC, while PHGDH expression was highest in HCA, followed by HCC, FC, and FA (P<0.001 and P=0.041 respectively, **Table 3** and **Figure 2**).

Correlations between the expression of serine/glycine-related proteins and clinicopathologic factors in follicular carcinoma

Next, we investigated correlations between the expression of serine/glycine-related proteins and clinicopathologic factors in FC. SHMT1 negativity was associated with extrathyroidal extension (P=0.019, **Figure 3**). No additional significant correlations between other serine/glycine-related proteins and clinicopathologic factors were found.

The impact of expression of serine/glycine-related proteins on prognosis

Using Cox-proportional hazard analysis, we investigated the prognostic significance of serine/glycine-related protein expression in FC and HCC. Among serine/glycine-related proteins, PSAT1 negativity was associated with shorter overall survival (OS) (P<0.001, **Table 4**).

Discussion

In this study, we investigated the expression of the serine/glycine-related proteins PHGDH,

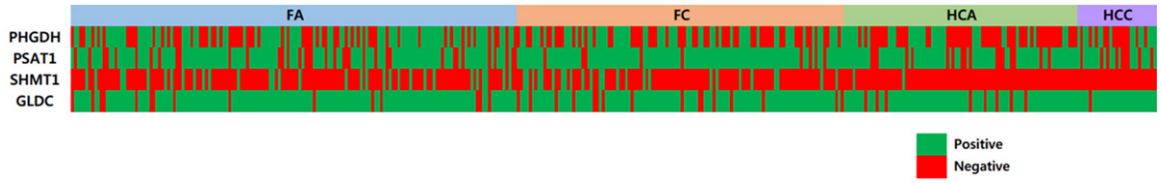

Figure 1. Heat map of serine and glycine metabolism-related proteins in FN and HCN. The expression of PHGDH and SHMT1 was higher in HCN compared to FN. FA, follicular adenoma; FC, follicular carcinoma; HCA, Hürthle cell adenoma; HCC, Hürthle cell carcinoma; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase-1; SHMT1, serine hydroxymethyltransferase-1; GLDC, glycine decarboxylase.

Table 3. Expression of serine and glycine metabolism-related proteins in follicular adenoma, follicular carcinoma, Hürthle cell adenoma, and Hürthle cell carcinoma

Parameters	Follicular neoplasm n=265 (%)		Hürthle cell neoplasm n=107 (%)		*p*-value
	FA n=153(%)	FC n=112(%)	HCA n=80(%)	HCC n=27(%)	
PSAT1					0.002
Negative	116 (75.8%)	103 (92.0%)	63 (78.8%)	18 (66.7%)	
Positive	37 (24.2%)	9 (8.0%)	17 (21.2%)	9 (33.3%)	
GLDC					0.387
Negative	138 (90.2%)	96 (85.7%)	72 (90.0%)	26 (96.3%)	
Positive	15 (9.8%)	16 (14.3%)	8 (10.0%)	1 (3.7%)	
SHMT1					<0.001
Negative	31 (20.3%)	25 (22.3%)	2 (2.5%)	0 (0.0%)	
Positive	122 (79.7%)	87 (77.7%)	78 (97.5%)	27 (100.0%)	
PHGDH*					0.041
Negative	89 (58.2%)	58 (51.8%)	31 (38.8%)	14 (51.9%)	
Positive	63 (41.2%)	54 (48.2%)	49 (61.2%)	13 (48.1%)	

FA, follicular adenoma; FC, follicular carcinoma; HCA, Hürthle cell adenoma; HCC, Hürthle cell carcinoma; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase-1; SHMT1, serine hydroxymethyltransferase-1; GLDC, glycine decarboxylase. *Immunohistochemistry was not carried out in one case.

PSAT1, SHMT1, and GLDC in HCN and FN. Compared to FN, expression of some serine/glycine-related proteins was higher in HCN. In a previous publication, serine/glycine-related protein expression was evaluated according to thyroid cancer subtype [18], but ours is the first study to evaluate such expression in FN and HCN. Pathak et al. evaluated glycolysis using 18F-FDG PET/CT in FA and HCA and found that FDG uptake was higher in HCA compared to FA, suggesting that glycolysis is more active in HCA than FA [16, 19]. Thus, we predicted that we would find differences in metabolism between HCN and FN. Our observations of differences in serine/glycine-related protein expression between HCN and FN could be explained by several mechanisms.

Genomic dissection has shown that HCC shows activated PIK3CA-Akt-mTOR and Wnt/β-catenin pathways compared to other tumors [14]. Mutation in PIK3CA regulates glycolysis via mobilization of aldolase from the actin cytoskeleton [20] and was related to aerobic glycolysis in an epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma cell line [21]. In addition, Wnt/β-catenin pathways direct toward glycolysis and angiogenesis in colon cancer [22], and overexpression of glycolysis-related molecules is dependent on β-catenin mRNA expression level in solid pseudopapillary neoplasms of the pancreas [23]. Therefore, the activated PIK3CA-Akt-mTOR and Wnt/β-catenin pathways could influence the serine/glycine metabolic pathway, which is a glycolytic intermediate. Among the molecular features of HCN, the TERT C228T promoter mutation is reported to be common [15], with a very short telomere length [15, 24]. Several reports indicate that down-regulation of genes involved in the glycolytic pathway leads to TERT knockdown, suggesting that TERT is directly related to cancer cell metabolism via glycolysis [25, 26]. Accordingly, it seems feasible that serine/glycine-related protein expression in HCN is increased. However, the underlying mechanisms between the PIK3CA-Akt-mTOR pathway, Wnt/β-catenin pathway, TERT C228T promoter mutation, and increased expression of serine/glycine-related proteins are not well understood.

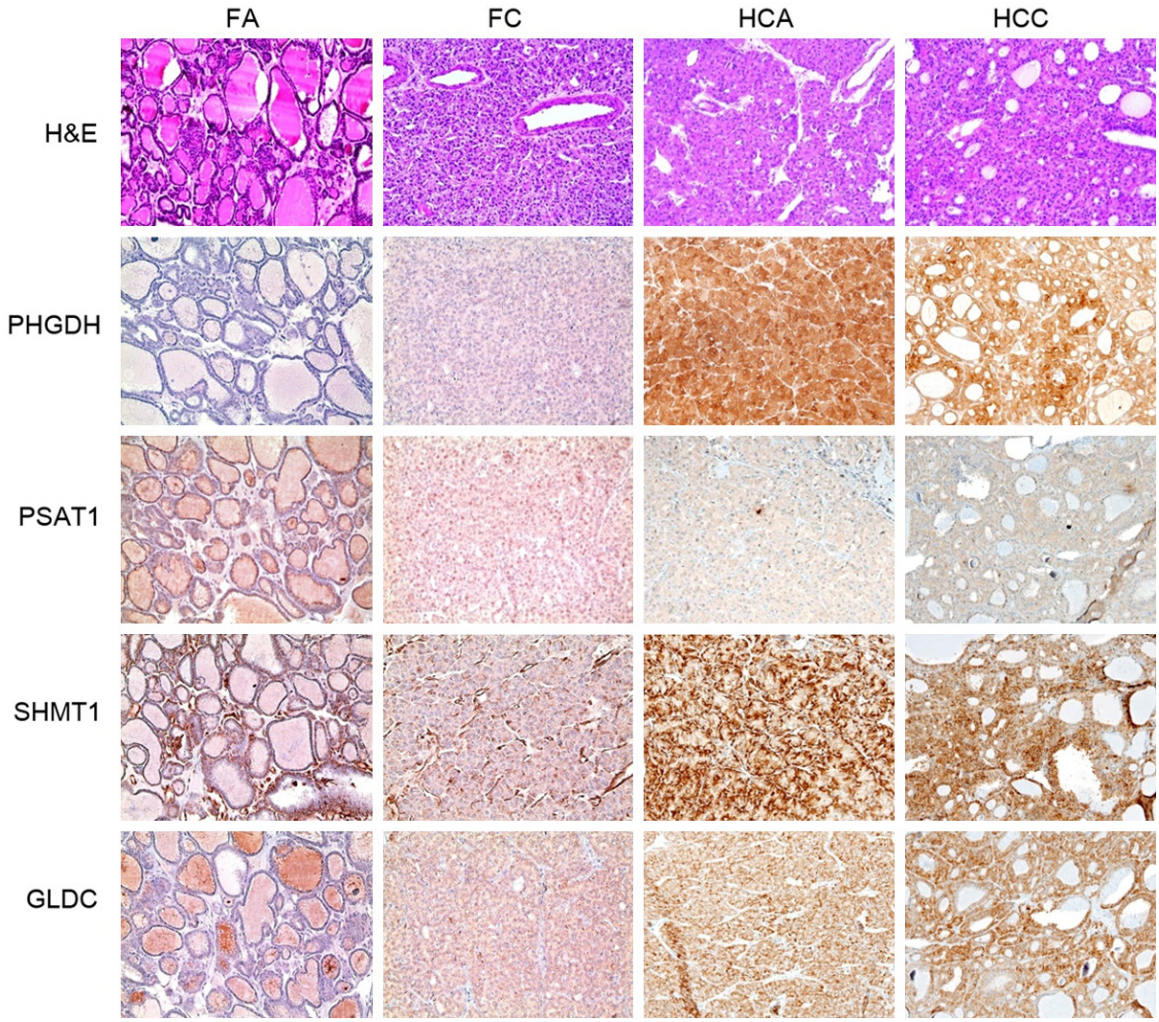

Figure 2. Expression of serine and glycine metabolism-related proteins in FA, FC, HCA, and HCC. SHMT1 expression was highest in HCC, followed by HCA, FA, and FC, while PHGDH expression was highest in HCA, followed by HCC, FC, and FA. FA, follicular adenoma; FC, follicular carcinoma; HCA, Hürthle cell adenoma; HCC, Hürthle cell carcinoma; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase-1; SHMT1, serine hydroxymethyltransferase-1; GLDC, glycine decarboxylase.

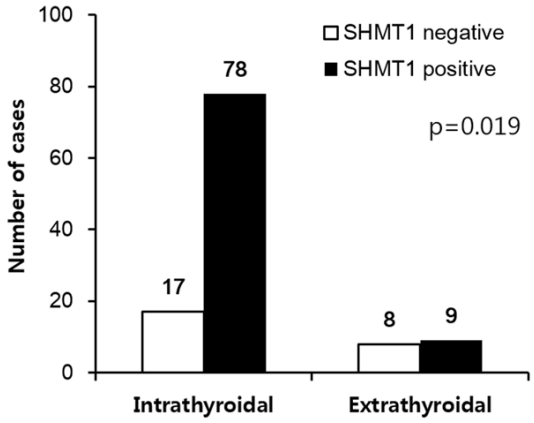

Figure 3. Correlations between the expression of serine and glycine metabolism-related proteins and clinicopathologic factors in FC. SHMT1 negativity was associated with extrathyroidal extension. No additional significant correlations between other serine/glycine-related proteins and clinicopathologic factors were found. FC, follicular carcinoma; SHMT1, serine hydroxymethyltransferase-1.

Notably, SHMT1 negativity was related to extrathyroidal extension in FC. In a previous study in high grade serous ovarian cancer, SHMT1 was related to cancer growth, cancer cell migration, and tumorigenesis in murine models [27]. Furthermore, miR-198, a targeted inhibitor of SHMT1, has been shown to hamper cancer cell proliferation and prevent cell apoptosis in lung adenocarcinoma [28]. Collectively, these findings suggest that SHMT1 expression is related to aggressive tumor biology. However, in

Table 4. Univariate analysis of the influence of serine and glycine metabolism-related protein expression in follicular carcinoma and Hürthle cell carcinoma on disease-free survival and overall survival

Parameter	Number of patients (n=139)/death	Disease-free survival		Overall survival	
		Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value
PSAT1					
Negative	121/5	n/a	n/a	35.1 (30.5-39.7)	<0.001
Positive	18/0	n/a	n/a	57.4 (45.6-69.2)	
GLDC					0.078
Negative	122/3	n/a	n/a	38.2 (33.5-43.0)	
Positive	17/2	n/a	n/a	36.1 (21.8-50.5)	
SHMT1					0.948
Negative	25/1	n/a	n/a	38.2 (31.7-44.8)	
Positive	114/4	n/a	n/a	37.9 (32.7-43.2)	
PHGDH					0.704
Negative	72/3	n/a	n/a	38.3 (31.8-44.8)	
Positive	67/2	n/a	n/a	37.7 (31.5-43.8)	

PSAT1, phosphoserine aminotransferase-1; GLDC, glycine decarboxylase; SHMT1, serine hydroxymethyltransferase-1; PHGDH, phosphoglycerate dehydrogenase.

this study, SHMT1 negativity was associated with extrathyroidal extension, which is a known poor prognostic factor in thyroid cancer. This difference in SHMT1 expression in various types of cancers requires further exploration in future studies.

Interestingly, PSAT1 negativity was associated with shorter OS in this study. In a previous study that evaluated serine metabolism-associated enzymes (PHGDH, PDK1, PDK2, PKM2, and PSAT) in colon cancer, PHGDH, PDK1, and PSAT expression was shown to be higher in cancer tissue compared to normal tissue [29]. Recently, a bioinformatics analysis found that PHGDH is a negative prognostic factor in breast cancer, but not lung cancer [30]. Therefore, the prognostic implications of serine/glycine-related protein expression could be different according to the subtype of cancer. In this study, patients with both FC and HCC were included for the analysis of patient prognosis. However, due to the heterogeneity of the included patients, our findings should be interpreted with caution.

The clinical implications of this study are that the serine/glycine metabolism pathway could be a possible therapeutic target in HCC. Currently, several inhibitors of SHMT1 and PHGDH are under development. Traditionally, methotrexate and 5-fluorouracil are antimetabolites targeting enzymes such as SHMT1 to exert anti-cancer effects. However, 3-bromopyruvate has recently shown promise as a novel anti-tumor agent by completely blocking SHMT1 by alkylating Cys204 residue of SHMT1 [31]. Furthermore, CBR-5884, which was developed as a PHGDH inhibitor, has been shown to inhibit cancer cell growth by blocking de novo serine synthesis even in cell lines with high serine biosynthetic activity [32]. Additionally, preclinical studies utilizing small molecules targeting catalytic sites of metabolic enzymes that include PSAT, and GLDC are underway [33]. Therefore, further experimental investigation regarding the impact of serine/glycine metabolism inhibition in HCC with high serine/glycine metabolic activity and preclinical studies are required.

In conclusion, the expression of serine/glycine-related proteins was different between FN and HCN. Among serine/glycine-related proteins, the expression of SHMT1 and PHGDH was higher in HCN compared to FN.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Clinicopathologic features of follicular carcinoma

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 2. Clinicopathologic features of Hürthle cell neoplasm

Parameters	Total N=107 (%)	Hürthle cell adenoma n=80 (%)	Hürthle cell carcinoma n=27 (%)	*p*-value
Age (years)				0.403
<45	45	36 (45.0%)	9 (33.3%)	
≥45	62	44 (55.0%)	18 (66.7%)	
Sex				0.027
Male	16	16 (20.0%)	0 (0.0%)	
Female	91	64 (80.0%)	27 (100.0%)	
Tumor size (cm)				0.007
≤2.0	74	62 (77.5%)	12 (44.4%)	
>2.0, ≤4.0	21	13 (16.3%)	8 (29.6%)	
>4.0	12	5 (6.2%)	7 (25.9%)	
Capsular invasion				<0.001
No	83	80 (100.0%)	3 (11.1%)	
Yes	24	0 (0.0%)	24 (88.9%)	
Vascular invasion				0.019
No	104	80 (100.0%)	24 (88.9%)	
Yes	3	0 (0.0%)	3 (11.1%)	
Tumor extension				<0.001
Intrathyroidal	101	80 (100.0%)	21 (77.8%)	
Extrathyroidal	6	0 (0.0%)	6 (22.2%)	
LN metastasis				n/a
No	107 (100.0)	80 (100.0)	27 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Distant metastasis				n/a
No	107 (100.0)	80 (100.0)	27 (100.0)	
Yes	0 (0.0)	0 (0.0)	0 (0.0)	