Interaction of H3K27me3 and Glutaminase 1 Expression on Breast Cancer Prognosis by Menopausal Status: a Cohort Study

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Abstract

Background: Glutaminase 1 (GLS) is a potential therapeutic target for breast cancer; although GLS inhibitors have been developed, only a few subjects responded well to the therapy. Considering that the expression of trimethylation of histone H3 lysine 27 (H3K27me3) and menopausal status have been closely linked to the role of GLS, we tried to examine the modification effects of H3K27me3 and menopausal status on GLS to breast cancer prognosis, which would be helpful to identify the more suitable patients to the GLS inhibitors.

Methods: Data for 963 women diagnosed with primary invasive breast cancer between 2008 and 2015 were analyzed. H3K27me3 and GLS expression in tumors were evaluated with tissue microarrays by immunohistochemistry. Hazard ratios (*HRs*) and their 95% confidence intervals (*CI*s) for overall survival (OS) and progression-free survival (PFS) were estimated using univariable and multivariable Cox regression models. The interaction was assessed on multiplicative scale by stratification analysis.

Results: After a median follow-up of 70.6 months (interquartile range: 45.6-103.9), we confirmed the association between H3K27me3 and both outcomes (*HR* = 0.57, 95% *CI*: 0.37-0.86 for OS; *HR* = 0.66, 95% *CI*: 0.48-0.91 for PFS) and found that the prognostic roles of GLS were not statistically significant in the overall patients. There was a beneficial prognostic effect of GLS expression on OS for those with low H3K27me3 level (*HR* = 0.50, 95% *CI*: 0.20-1.28) but an adverse prognostic effect for those with high H3K27me3 level (*HR* = 3.90, 95% *CI*: 1.29-11.78) among premenopausal women, and the interaction was significant ($P_{\text{interaction}} = 0.003$). Similar pattern was further observed for PFS (*HR* = 0.44, 95% *CI*: 0.20-0.95 for low H3K27me3 level, *HR* = 1.35, 95% *CI*: 0.74-2.48 for high H3K27me3 level, $P_{\text{interaction}} = 0.024$). The interaction didn't occur among postmenopausal women.

Conclusions: This study revealed the modification effects of H3K27me3 and menopausal status on GLS to breast cancer prognosis, which would help optimize the medication strategies related to GLS inhibitors.

Background

Glutaminase 1 (GLS) is a key enzyme in glutamine catabolism which is critical for the proliferation of various tumors^[1-3]. Many studies have found that GLS overexpression was associated with the poor prognostic characteristics of breast cancer^[4-6]. Thereafter, GLS inhibitors have emerged as a therapeutic avenue for breast cancer^[7-9]. However, the results of clinical trials were not promising^[10]: the objective response rates were only 22% in the Phase I study and 6% in the Phase II study^[11, 12]. Actually, the findings of the association between GLS expression and breast cancer prognosis were not consistent. For example, a higher expression of GLS was observed to be related to a better prognosis^[13]. Therefore, we hypothesize that the effects of GLS or the inhibitors on breast cancer may be affected by other factors.

It was found that breast cancer cells which resisted GLS inhibitors mobilized more fatty acids into mitochondria for oxidation^[14]. Moreover, the increased fatty acid catabolism was associated with a

decrease of trimethylation of histone H3 lysine 27 (H3K27me3) ^[15, 16] which was also an independent prognostic factor for breast cancer ^[17, 18]. Furthermore, many studies have found that the decreased level of H3K27me3 suppressed the transcription of oncogene MYC which was essential for the expression of GLS ^[19, 20]. Thus, the relationship between GLS and H3K27me3 was intriguing, which may affect the prognostic role of GLS on breast cancer.

In addition, it was found that women with high progesterone level have higher level of H3K27me3 ^[21, 22] and estrogen has the effect of up-regulating GLS ^[23], which suggested that the interaction between GLS and H3K27me3 may be differentiated by menopause. Therefore, in the present study, we examined the modification effect of H3K27me3 on GLS to breast cancer prognosis by menopausal status.

Materials And Methods

Study population

A total of 1062 female patients with pathologically diagnosed primary invasive breast cancer and > 1 cm of tumor size in diameter between January 2008 and December 2015 were recruited from the Cancer Center of Sun Yat-sen University in Guangzhou, China. Patients with metastatic tumor and missing information of H3K27me3 and GLS (*N*= 87) were excluded. Most (98.8%) of the included patients were successfully followed up until Dec 31, 2019. This study was approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University. Informed consent was obtained from each participant.

Baseline data collection

Information on demographic and clinicopathologic characteristics was collected at diagnosis using structured questionnaire and from patients' medical records, including age, menopausal status, body mass index (BMI), family history of breast cancer, clinical stage, histological grade, estrogen receptor (ER), progesterone receptor (PR), Human epidermal growth factor receptor 2 (HER2) status and proliferation index factor Ki67 (Ki67) etc. The definition of ER, PR, and HER2 status was described in detail previously ^[24].

Tissue microarray and Immunohistochemistry

The expression levels of H3K27me3 and GLS were evaluated with tissue microarrays (TMAs) by immunohistochemistry (IHC). TMAs were constructed as previously described ^[25]. The TMAs were baked at 60°C for 2 hours and then dewaxed with xylene and ethanol. Then antigen retrieval was accomplished using EDTA (PH 9.0) in super-pressure kettle and endogenous peroxide was blocked using 3% H₂O₂. Antigen-antibody reactions for H3K27me3 and GLS were performed separately. For H3K27me3, slides were incubated in mouse monoclonal to H3K27me3 [mAbcam 6002]-ChIP Grade (ab6002, diluted 1:100, Abcam) and then labeled with the EnVision Detection System (Peroxidase/DAB, Rabbit/Mouse) (Dako K5007). For GLS, slides were incubated in rabbit monoclonal to GLS [EP7212] (ab156876, diluted 1:100,

Abcam). Then slides were developed by diaminobenzidine (DAB) and counterstained by hematoxylin. These slides were finally dehydrated and mounted.

IHC stained sections were digitally imaged using Panoramic Scanner and CaseViewer software. IHC staining was analyzed by an experienced pathologist and scored for staining intensity (0-no staining, 1-weak, 2-moderate and 3-strong) and percentage of tumor cell staining (0-100). IHC scoring was done by H-score which involved the product of both the intensity of staining and the percentage of positive cells. The range of possible scores were thus 0-300. To avoid the observation variability, the mean value of duplicate scores was adapted for further analysis.

Follow up and Outcomes

Patients were followed up by phone calls or out-patient visits every 3 months in the first year, every 6 months in the second and third year after diagnosis and annually thereafter. Outcomes of interest were overall survival (OS) and progression-free survival (PFS). OS was defined as the time from diagnosis to death and PFS was the time from diagnosis to disease progression including recurrence, metastasis, and death. Survival status was censored at the latest follow-up date or Dec 31, 2019.

Statistical analysis

The expressions of H3K27me3 and GLS were treated as binary variables. The optimal cut-off value of H3K27me3 was determined by the minimum *P* value from log-rank chi-square statistics based on PFS using the X-tile 3.6.1 software (Yale University, New Haven, CT, USA) [26]. For GLS, in fact, nearly half (44.2%) of the H-score of GLS was 0. Therefore, the cut-off value of GLS was determined by the H-score = 0 or not. Next, GLS H-score = 0 was considered negative and GLS H-score > 0 was considered positive. Frequency distribution was used to compare demographic and clinicopathologic characteristics according to H3K27me3 and GLS category. Kaplan-Meier method was used to estimate the 5-year survival. Cox proportional hazard model was used to estimate hazard ratios (*HRs*) and their 95% confidence intervals (*CI*s) for the associations between demographic and clinicopathologic characteristics and survival (OS and PFS) and the associations between H3K27me3 and GLS and survival.

The interaction between H3K27me3 and GLS on survival was evaluated on the multiplicative scale. *HRs* (95% *CI*s) for the association between GLS and survival within H3K27me3 strata were calculated. Furthermore, the interaction was separately tested in premenopausal and postmenopausal patients. All the analyses were conducted using R 3.6.3 and a two-sided *P*-value below 0.05 was considered as statistical significance.

Results

Demographic and clinicopathological characteristics and the associations with H3K27me3 and GLS expression and

breast cancer prognosis

Of 975 eligible women, 963 (98.8%) were included in the statistical analysis after excluding women with loss of follow-up (Fig. 1). The median age at diagnosis was 48 years (interquartile range: 42–56). More than half (58.0%) of the women were premenopausal and 56.0% of them had a BMI between 18.5 and 23.9 kg/m². The majority of the women were diagnosed with low histological grade (grade I/II: 73.1%), early clinical stage (stage I/II: 71.7%), ER-positive (73.2%), PR-positive (72.2%), or HER2-negative (66.5%) (Table 1).

Table 1
Demographic and clinicopathological characteristics and the associations with H3K27me3 and GLS [N (%)]

Characteristics	Total N= 963	H3K27me3		GLS			
		≤ 175 (n = 274)	>175 (n = 689)	P value*	negativity (n = 426)	positivity (n = 537)	P value*
Age (years)				0.339			0.687
≤ 35	095 (09.9)	033 (12.0)	062 (09.0)		046 (10.8)	049 (09.1)	
36–50	460 (47.8)	130 (47.4)	330 (47.9)		201 (47.2)	259 (48.2)	
> 50	408 (42.4)	111 (40.5)	297 (43.1)		179 (42.0)	229 (42.6)	
Menopause				0.069			0.691
Pre-	534 (58.0)	166 (62.6)	368 (56.1)		243 (58.7)	291 (57.4)	
Post-	387 (42.0)	099 (37.4)	288 (43.9)		171 (41.3)	216 (42.6)	
Missing	042 (00.0)	009 (00.0)	033 (00.0)		012 (00.0)	030 (00.0)	
Age at menarche				0.508			0.102
≤ 12	079 (08.5)	025 (09.4)	054 (08.1)		042 (10.1)	037 (07.1)	
> 12	853 (91.5)	240 (90.6)	613 (91.9)		372 (89.9)	481 (92.9)	
Missing	031 (00.0)	009 (00.0)	022 (00.0)		012 (00.0)	019 (00.0)	
BMI (kg/m ²)				0.687			0.398
< 18.5	050 (05.5)	017 (06.5)	033 (05.1)		019 (04.7)	031 (06.1)	
18.5–23.9	512 (56.0)	146 (55.7)	366 (56.1)		223 (54.8)	289 (57.0)	
≥ 24.0	352 (38.5)	099 (37.8)	253 (38.8)		165 (40.5)	187 (36.9)	
Missing	049 (00.0)	012 (00.0)	037 (00.0)		019 (00.0)	030 (00.0)	

Characteristics	Total <i>N</i> = 963	H3K27me3		<i>P</i> value*	GLS		<i>P</i> value*
		≤ 175 (<i>n</i> = 274)	>175 (<i>n</i> = 689)		negativity (<i>n</i> = 426)	positivity (<i>n</i> = 537)	
Family history				0.421			0.638
No	842 (89.7)	241 (90.9)	601 (89.2)		377 (90.2)	465 (89.3)	
Yes	097 (10.3)	024 (09.1)	073 (10.8)		041 (09.8)	056 (10.7)	
Missing	024 (00.0)	009 (00.0)	015 (00.0)		008 (00.0)	016 (00.0)	
Parity				0.326			0.228
0	036 (03.9)	014 (05.2)	022 (03.3)		011 (02.7)	025 (04.8)	
1–2	660 (70.8)	183 (68.5)	477 (71.7)		293 (71.1)	367 (70.6)	
≥ 3	236 (25.3)	070 (26.2)	166 (25.0)		108 (26.2)	128 (24.6)	
Missing	031 (00.0)	007 (00.0)	024 (00.0)		014 (00.0)	017 (00.0)	
Histological grade				< 0.001			< 0.001
I/II	648 (73.1)	158 (63.2)	490 (77.0)		304 (79.4)	344 (68.4)	
III	238 (26.9)	092 (36.8)	146 (23.0)		079 (20.6)	159 (31.6)	
Missing	077 (00.0)	024 (00.0)	053 (00.0)		043 (00.0)	034 (00.0)	
Tumor size (cm)				0.175			0.471
≤ 2	294 (30.6)	075 (27.4)	219 (31.8)		135 (31.8)	159 (29.6)	
> 2	668 (69.4)	199 (72.6)	469 (68.2)		290 (68.2)	378 (70.4)	
Missing	001 (00.0)	000 (00.0)	001 (00.0)		001 (00.0)	000 (00.0)	
Nodal status				0.863			0.644
Negative	433 (45.0)	122 (44.5)	311 (45.1)		188 (44.1)	245 (45.6)	

Characteristics	Total <i>N</i> = 963	H3K27me3		<i>P</i> value*	GLS		<i>P</i> value*
		≤ 175 (<i>n</i> = 274)	>175 (<i>n</i> = 689)		negativity (<i>n</i> = 426)	positivity (<i>n</i> = 537)	
Positive	530 (55.0)	152 (55.5)	378 (54.9)		238 (55.9)	292 (54.4)	
Clinical stage				0.623			0.483
I	174 (18.1)	046 (16.8)	128 (18.6)		072 (16.9)	102 (19.0)	
II	516 (53.6)	145 (52.9)	371 (53.9)		237 (55.8)	279 (52.0)	
III	272 (28.3)	083 (30.3)	189 (27.5)		116 (27.3)	156 (29.1)	
Missing	001 (00.0)	000 (00.0)	001 (00.0)		001 (00.0)	000 (00.0)	
ER				< 0.001			< 0.001
Negative	248 (26.8)	113 (42.6)	135 (20.5)		087 (21.1)	161 (31.5)	
Positive	676 (73.2)	152 (57.4)	524 (79.5)		326 (78.9)	350 (68.5)	
Missing	039 (00.0)	009 (00.0)	030 (00.0)		013 (00.0)	026 (00.0)	
PR				< 0.001			0.087
Negative	257 (27.8)	102 (38.3)	155 (23.6)		103 (25.0)	154 (30.1)	
Positive	667 (72.2)	164 (61.7)	503 (76.4)		309 (75.0)	358 (69.9)	
Missing	039 (00.0)	008 (00.0)	031 (00.0)		014 (00.0)	025 (00.0)	
HER2				0.140			0.512
Negative	640 (66.5)	169 (61.7)	471 (68.4)		291 (68.3)	349 (65.0)	
Equivocal	080 (08.3)	026 (09.5)	054 (07.8)		035 (08.2)	045 (08.4)	
Positive	243 (25.2)	079 (28.8)	164 (23.8)		100 (23.5)	143 (26.6)	
Ki67				< 0.001			< 0.001

Characteristics	Total <i>N</i> = 963	H3K27me3		<i>P</i> value*	GLS		<i>P</i> value*
		≤ 175 (<i>n</i> = 274)	> 175 (<i>n</i> = 689)		negativity (<i>n</i> = 426)	positivity (<i>n</i> = 537)	
≤ 14%	208 (28.7)	031 (14.6)	177 (34.6)		118 (36.4)	090 (22.5)	
> 14%	516 (71.3)	181 (85.4)	335 (65.4)		206 (63.6)	310 (77.5)	
Missing	239 (00.0)	062 (00.0)	177 (00.0)		102 (00.0)	137 (00.0)	
TNBC				< 0.001			0.031
No	795 (92.7)	204 (84.0)	591 (96.1)		364 (94.8)	431 (90.9)	
Yes	063 (07.3)	039 (16.0)	024 (03.9)		020 (05.2)	043 (09.1)	
Missing	105 (00.0)	031 (00.0)	074 (00.0)		042 (00.0)	063 (00.0)	
* for chi-square test							
Abbreviations: BMI = body mass index, ER = estrogen receptor, GLS = glutaminase 1, HER2 = human epidermal growth factor receptor 2, Ki67 = proliferation index factor Ki67, PR = progesterone receptor, TNBC = triple-negative breast cancer							

The optimal cut-off value of H3K27me3 H-score was 175 according to the X-tile plot (see Additional file 1, Supplementary Fig. 1). A great part (71.5%) of the women had the H-score > 175 of H3K27me3 and the percentage of GLS negativity was 44.2%. For H3K27me3, women with the H-score ≤ 175 were more likely to be premenopausal and have grade III, ER-negative, PR-negative and Ki67 > 14% tumors than the subjects with H-score > 175. For GLS, women with GLS negativity were more likely to have grade I/II, ER-positive and Ki67 ≤ 14% tumors than the subjects with GLS positivity (Table 1). Univariable analysis showed that age, BMI, histological grade, clinical stage and ER were associated with OS and clinical stage and ER were associated with PFS (see Additional file 2, Supplemental Table 1).

Prognostic effects of H3K27me3 and GLS on breast cancer

Of the 963 eligible women, 102 died and 187 experienced disease progression with a median follow-up time of 70.6 months (interquartile range: 45.6-103.9). Five-year OS rate and PFS rate were 91.4% and 84.9%, respectively. A consistent relation between H3K27me3 and survival (OS and PFS) was observed from both univariable and multivariable analyses (Table 2). After adjustment for confounders, women with H3K27me3 H-score > 175 (*HR* = 0.57, 95% *CI*: 0.37–0.86) had a better OS compared to H-score ≤ 175.

A similar pattern of association was observed for PFS ($HR = 0.66$, 95% CI : 0.48–0.91) (Table 2). The prognostic roles of GLS were not statistically significant in the overall patients (Table 2).

Table 2
Hazard ratios for the association between H3K27me3 and GLS and the outcomes

Markers	Total (%)	Events (%)	Crude <i>HR (95%CI)</i>	Adjusted <i>HR (95%CI)</i> ^a
H3K27me3				
OS				
≤ 175	274 (28.5)	045 (44.1)	1.00 (reference)	1.00 (reference)
> 175	689 (71.5)	057 (55.9)	0.46 (0.31,0.68)	0.57 (0.37,0.86)
PFS				
≤ 175	274 (28.5)	072 (38.5)	1.00 (reference)	1.00 (reference)
> 175	689 (71.5)	115 (61.5)	0.57 (0.42,0.76)	0.66 (0.48,0.91)
GLS				
OS				
negative	426 (44.2)	039 (38.2)	1.00 (reference)	1.00 (reference)
positive	537 (55.8)	063 (61.8)	1.25 (0.84,1.86)	1.06 (0.69,1.62)
PFS				
negative	426 (44.2)	079 (42.2)	1.00 (reference)	1.00 (reference)
positive	537 (55.8)	108 (57.8)	1.05 (0.79,1.41)	0.92 (0.68,1.26)
^a Adjusted for age at diagnosis, clinical stage, histological grade, ER status Abbreviations: CI = confidence interval, GLS = glutaminase 1, HR = Hazard ratio, OS = overall survival, PFS = progression-free survival Bold characters indicate statistically significant result				

Modification effect

The interaction between H3K27me3 and GLS on OS ($P_{\text{interaction}} = 0.003$) and PFS ($P_{\text{interaction}} = 0.024$) was statistically significant among premenopausal women (Table 3). GLS positivity was significantly associated with a poorer OS compared to GLS negativity ($HR = 3.90$, 95% CI : 1.29–11.78) in patients with H3K27me3 H-score > 175, while it was related to a better OS ($HR = 0.50$, 95% CI : 0.20–1.28) in patients

with H3K27me3 H-score ≤ 175 among premenopausal women. Similar pattern was also observed for PFS.

Table 3

Modification effect of H3K27me3 on the association between GLS and outcomes by menopausal status

H3K27me3	GLS	Pre-menopause			Post-menopause		
		Events / Total	HR (95%CI) a	P _{interaction}	Events / Total	HR (95%CI) a	P _{interaction}
OS				0.003			0.730
≤ 175	negative	13/079	1.00 (reference)		08/049	1.00 (reference)	
	positive	12/087	0.50 (0.20,1.28)		09/050	0.59 (0.17,2.09)	
> 175	negative	04/164	1.00 (reference)		13/122	1.00 (reference)	
	positive	19/204	3.90 (1.29,11.78)		20/166	1.00 (0.48,2.06)	
PFS				0.024			0.720
≤ 175	negative	22/079	1.00 (reference)		14/049	1.00 (reference)	
	positive	18/087	0.44 (0.20,0.95)		15/050	0.76 (0.32,1.82)	
> 175	negative	20/164	1.00 (reference)		21/122	1.00 (reference)	
	positive	34/204	1.35 (0.74,2.48)		34/166	1.09 (0.62,1.91)	
^a Adjusted for age at diagnosis, clinical stage, histological grade, ER status							
Abbreviations: CI = confidence interval, GLS = glutaminase 1, HR = Hazard ratio, OS = overall survival, PFS = progression-free survival							
Bold characters indicate statistically significant result							

Discussion

In the present study, we found that a higher GLS expression level was associated with more aggressive characteristics of breast cancer, such as higher histological grade, ER negative and Ki67 overexpression. There was also an interaction between H3K27me3 and GLS on breast cancer prognosis, specifically among premenopausal women. Compared with the GLS negativity, GLS positivity was associated with a

protective effect on the survival of patients with a lower H3K27me3 expression, while it was associated with an adverse effect on the survival of patients with a higher level of H3K27me3 expression among premenopausal women. Our results suggested that the prognostic effects of GLS on breast cancer may depend on the H3K27me3 expression of the tumor and the menopausal status of patients.

In consistent with our study, several previous studies have also found that the higher GLS expression was associated with the poor prognostic characteristics but not associated with the outcomes of breast cancer [5, 6, 27]. These characteristics, such as histological grade and ER status, were usually associated with breast cancer prognosis [28, 29], suggesting that GLS expression was literally related to the prognosis. However, when we adjusted these characteristics, the association strength between GLS expression and the prognosis was significantly decreased; we found that these characteristics were correlated to GLS expression and this phenomenon was probably caused by collinearity.

It was interesting and pragmatic that there was an opposite prognostic effect of GLS among premenopausal women with high and low levels of H3K27me3 expression. Previous studies have found that a low level of H3K27me3 expression was associated with an increased fatty acid catabolism [15, 16]. Considering that fatty acids were utilized as an alternative energy source after glutamine deprivation in breast cancer cells [30], the low level of H3K27me3 suggested the deprivation of glutamine and the effect of GLS on catalyzing glutamine catabolism (which was recognized to be related to a poor prognosis) would be depressed, inducing a better prognosis of breast cancer. In turn, among women with a higher H3K27me3 expression, GLS would largely play its role with sufficient glutamine and led a poor prognosis. Moreover, it has been found that the breast cancer cells which resisted GLS inhibitors mobilized more fatty acids to catabolize [14], which also supported our hypothesis. In addition, compared with postmenopausal women, premenopausal women had a significantly lower serum concentration of glutamine [31], which would accelerate the deprivation of glutamine, supporting that the interaction between H3K27me3 and GLS was evident among premenopausal women. Furthermore, it may also partially explain why the interaction only existed in premenopausal women that the overall metabolism capacity of premenopausal women was higher than postmenopausal women [32, 33].

Our study has several limitations that need to be taken into consideration. First, we were unable to separately evaluate the three isoforms of GLS (KGA, GAC and GAM) expression [6, 34] and their associations with breast cancer prognosis. However, these three isoforms of GLS play the same role in breast cancer, so it is feasible to assess its relationship with breast cancer prognosis using total GLS protein expression, as has been done in previous literatures [1, 6]. Second, only patients with tumor > 1 cm were included, which may lead to selective bias. However, GLS expression was independent of tumor size in this study and the selection may not affect our findings. Finally, we didn't collect the information of treatment which was associated with the outcomes. However, since the treatment was determined according to the clinicopathological characteristics, and adjustment of these characteristics in the analysis was able to largely control the confounding effects of the treatment.

Conclusions

In summary, this study firstly demonstrated that the prognostic roles of GLS expression on breast cancer were depended on the level of H3K27me3 expression and this interaction occurred only if the patients were premenopausal rather than postmenopausal. When the premenopausal women express a low H3K27me3 level, the overexpression of GLS may be a protective factor according to this study, thus, these patients may not be suitable for the treatment of GLS inhibitors. In turn, GLS inhibitors may be more effective for premenopausal women with the high H3K27me3 expression. Therefore, it is necessary to consider the level of H3K27me3 expression and the menopausal status when studying the application of GLS inhibitors.

Abbreviations

BMI: Body mass index

CI: Confidence interval

DAB: Diaminobenzidine

ER: Estrogen receptor

GLS: Glutaminase 1

H3K27me3: Trimethylation of histone H3 lysine 27

HER2: Human epidermal growth factor receptor 2

HR: Hazard ratio

IHC: Immunohistochemistry

Ki67: Proliferation index factor Ki67

OS: Overall survival

PFS: Progression-free survival

PR: Progesterone receptor

TMA: Tissue microarray

TNBC: Triple-negative breast cancer.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of School of Public Health, Sun Yat-sen University. All participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MZ, ZFR, and QXC designed and directed the study, wrote and/or revised the manuscript. YZY and YLL constructed the TMAs. YZY contributed to the IHC. ZJW, XFZ, JXG, and LYT contributed to digital imaging of IHC-stained sections and the assessment of immunohistochemical expression. MZ, QXC, ZZL, ZYH, and YLL contributed to clinical data collection and curation. MZ, QXC, ZZL, and ZYH participated in the statistical analysis plan and interpretation of results. ZFR provided administrative support and supervision for the study. All authors approved the final manuscript.

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Figures

Figure 1

Flow chart of the study cohort. Abbreviations: GLS = glutaminase

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