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The expressions and prognostic implications of Twist and E-cadherin in adenocarcinomas of the gastroesophageal junction and proximal gastric carcinoma

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Abstract

Twist and E-cadherin are crucial for the development of different types of cancer; however, their clinical significance in adenocarcinoma of the gastroesophageal junction (AGE) remains unknown. Here, we investigated the correlation between the expression of Twist and E-cadherin and their impact on the clinical outcomes and prognosis of patients with AGE and proximal gastric carcinoma (PGC).

Using immunohistochemistry, we determined the expression of Twist and E-cadherin in the tissue samples of patients with AGE and PGC. The correlation of the expression of Twist and E-cadherin with the clinicopathological factors was assessed by using the chi-square test, Fisher exact test, and non-parametric Mann–Whitney *U* test. The Kaplan–Meier method along with the log-rank test and Cox proportional-hazards model were used to evaluate the correlation of Twist and E-cadherin expression with the overall survival (OS) of patients.

Overall, 94 patients with AGE (n = 45, 47.87%) or PGC (n = 49, 52.13%) who underwent primary tumor resection were included in this study. The median follow-up period was 40.5 months. We observed a significant difference in the smoking status ($P < .001$) and differentiation grade ($P = .004$) between patients with AGE and PGC. There was a significant association of a high Twist expression with T stage (only in PGC, $P = .008$), lymph node metastasis (AGE, $P = .075$; PGC, $P = .051$), and advanced pathological stages (AGE, $P = .019$; PGC, $P = .006$). A low E-cadherin expression showed similar results; however, it was not significantly associated with the advanced pathological stages of AGE ($P = .372$). A low E-cadherin expression was significantly associated with a low differentiation grade of AGE ($P = .002$). In addition, a significant inverse relationship was observed between Twist and E-cadherin expression. The Kaplan–Meier survival analysis and Cox regression analysis revealed that a high Twist expression and low E-cadherin expression were independent prognostic factors for short OS of patients with AGE or PGC.

A high Twist expression or low E-cadherin expression was associated with unfavorable clinicopathological factors and independently predicted short OS of patients with AGE or PGC.

Abbreviations: AGE = adenocarcinoma of the gastroesophageal junction, EMT = epithelial-to-mesenchymal transition, IRS = the intensity reactivity score, OS = overall survival, PGC = proximal gastric carcinoma, TNM = the tumor-node-metastasis.

Keywords: E-cadherin, gastric carcinoma, gastroesophageal junction, prognosis, Twist

1. Introduction

There has been an alarming increase in the incidence of mortality due to adenocarcinoma of the gastroesophageal junction (AGE) worldwide over the last few decades.[1–3] Smoking, obesity, and gastroesophageal reflux disease are significant risk factors for AGE and may account for a substantial fraction of the total disease burden. Although the incidence and mortality rate of gastric cancer are steadily decreasing, it remains the second most common cause of cancer death worldwide. Several studies have investigated whether the anatomic location of the carcinomas of the upper gastrointestinal tract is associated with specific predictors of clinical outcomes. Some investigators found differences in the gender predilection, TNM classification, differentiation grade, and prognosis, while others found similarities, particularly in the prognosis.[4–6]

E-cadherin mediates calcium-dependent cell-cell adhesion in epithelial tissue, which plays a pivotal role in epithelial cell behavior, tissue formation, and suppression of tumorigenicity and dissemination. However, low E-cadherin expression is the most common indicator of the onset of epithelial-mesenchymal transition (EMT) in many types of tumor[7]; the underlying molecular mechanism involves mutations, epigenetic and transcriptional silencing, increased endocytosis, and proteasomal degradation.[8] Many transcription factors have been shown to be involved; this includes the zinc finger protein Snail homologs

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and several basic helix-loop-helix transcription factors such as Twist, which interacts with the E-box element within the proximal region of the E-cadherin promoter, thereby affecting the repression of E-cadherin.[9]

Repression of E-cadherin or aberrant expression of Twist has been shown to be associated with unfavorable clinical features, including dedifferentiation, infiltrative growth, increased incidence of lymph node metastasis, and poor prognosis in several types of cancer[10–13]; however, their role in AGE remains unknown. Therefore, we aimed to investigate the correlation between the expression of Twist and E-cadherin and their impact on the clinical outcome and prognosis of patients with of AEG and PGC.

2. Methods

2.1. Patients and samples

Patients with AGE or PGC who underwent primary tumor resection in the Department of Gastrointestinal Surgery were included in this study. The study was approved by the Ethics Committee of the Weihai Municipal Hospital. Written informed consent was obtained from all the patients. Data on age, gender, smoking status, alcohol consumption, histological pattern, differentiation grade, pathologic staging, and survival status of the patients were obtained by reviewing the medical archive. The staging was determined according to the tumor-node-metastasis (TNM) classification of the Union for International Cancer Control; the histological type was determined according to the World Health Organization classification. Patients met the following inclusion criteria:

(I) diagnosed between January and December 2013;
(II) diagnosed with of AGE or PGC;
(III) underwent resection; and
(IV) did not receive adjuvant therapy before surgery.

We excluded patients who

(1) refused to undergo surgery, or subsequent treatment;
(2) were treated with non-curative intent;
(3) lost to follow-up; and (IV) refused to cooperate.

Resected specimens were assessed using immunohistochemistry.

2.2. Immunohistochemistry

Paraffin-embedded tissue samples were collected, cut into 3-μm-thick sections, and fixed on silicified slides. After deparaffinization and rehydration, the sections were heated in 0.01 mol/L saline citrate buffer at 96 to 98°C for 15 minutes to unmask antigens, then treated with 3% hydrogen peroxide for 15 minutes at room temperature to inactivate endogenous peroxidase, and finally incubated with 10% goat serum for 30 minutes at room temperature to block non-specific binding. The slides were then incubated overnight at 4°C with rabbit polyclonal antibodies for Twist (Abcam Biotechnology, clone H-81; diluted, 1:400) or mouse monoclonal antibodies for E-cadherin (ZSGB-BIO, clone 4A2C7; diluted, 1:100).

Streptavidin-peroxidase staining was performed according to the manufacturer instructions (Zymed Laboratories). The sections were then counterstained with hematoxylin before dehydration and mounting. For the negative control, the sections were incubated with phosphate-buffered saline instead of the antibodies.

2.3. Assessment of immunostaining

The immunostaining results were evaluated by using the intensity reactivity score (IRS); the staining intensity was considered negative (IRS = 0), weak (IRS = 1), moderate (IRS = 2), or strong (IRS = 3). Reactivity was evaluated by determining the fraction of positive cells in the entire tumor area; it was scored as 0 (<10%), 1 (11%–25%), 2 (26%–50%), 3 (51%–80%), or 4 (81%–100%). At least 10 fields were selected for each specimen. A multiplier for the intensity and fraction was calculated for each score, and the mean value of all the annotated scores was used for the analyses. The final scores were stratified into low expression (score, 0–7) or high expression (score, 8–12). The staining results were independently reviewed by 2 observers who were blinded to the clinical data of the patients. When there was any disagreement, a consensus was reached by re-reviewing the slides.

2.4. Statistical analysis

The chi-square test, Fisher exact test, and non-parametric Mann–Whitney *U* test were used to analyze the differences between the groups. Correlation between Twist and E-cadherin expression was analyzed using the Spearman rank test. The Kaplan–Meier method along with the log-rank test were used for the overall survival (OS) analysis. The Cox regression proportional-hazards model was used to estimate the impact of the prognostic factors on the OS. All tests were two-sided. *P* values $<.05$ were considered statistically significance. All statistical analyses were performed using the SPSS software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

3. Results

3.1. Patient and tumor characteristics

Of the 94 patients, 73 were men (77.66%) and the median age at diagnosis was 62 years (range: 29–79 years). Most of the patients were non-smokers (65.96%) but consumed alcohol (57.45%). Alcohol intake was analyzed by assessing the frequency in terms of the servings per week and the grams of ethanol intake per day, which was calculated by multiplying the frequency of consumption by the respective ethanol content. The duration of alcohol consumption was calculated from the age at which the patient started consuming alcohol to the age at which the patient was included in the study. A total of 36.17% of the tumors were well or moderately differentiated and 63.83% tumors were poorly differentiated. The median follow-up period for all patients was 40.5 months (range: 1–60 months). There were 45 (47.87%) patients with AGE and 49 (52.13%) with PGC. There were no significant differences in gender, age, drinking status, T stage, lymph node metastasis, M stage, and pathological stage between patients with AGE and PGC. In addition, PGC was significantly associated with poor differentiation. Interestingly, compared with patients with PGC, those with AGE were more commonly found to be smokers ($P < .001$). In addition, no significant difference in the OS was observed according to the Siewert types (log-rank

Table 1

Characteristics of patients.

		Entire cohort	AGE	PGC	*P*
Gender	Male	73	37	36	.309
	Female	21	8	13	
Age	median (range)	62 (29–79)	63 (45–78)	61 (29–79)	.157
Smoking status	Yes	32	24	8	<.001
	No	62	21	41	
Drinking status	Yes	54	26	28	.95
	No	40	19	21	
Differentiation	Well or moderate	34	23	11	.004
	Poor	60	22	38	
T stage	1	5	2	3	.830
	2	11	4	7	
	3	71	36	35	
	4	7	3	4	
Number of lymph node metastasis	Median (range)	4 (0–72)	3 (0–19)	4 (0–72)	.222
Metastasis	Yes	7	4	3	.706
	No	87	41	46	
Pathology stage	I	5	2	3	.109
	II	24	11	13	
	III	37	23	14	
	IV	28	9	19	
Expression of twist	High	55	26	29	.890
	Low	39	19	20	
Expression of E-cadherin	High	49	23	26	.850
	Low	45	22	23	

Age and number of lymph node metastasis were performed as median and range. *P* was used to compare clinicopathological characteristics for different tumor types, not including entire cohort.

$P = .094$; Figure 2A). The clinicopathological factors are shown in Table 1.

3.2. Correlation between Twist and E-cadherin expression

Twist was expressed in the cytoplasm and nucleus, while E-cadherin was expressed only in the cell membrane (Fig. 1); a significant inverse relationship between Twist and E-cadherin expression was observed in the entire cohort (correlation coefficient $= -0.476$, $P < .001$) and in the AGE and PGC groups (AGE, correlation coefficient $= -0.543$, $P < .001$; PGC, correlation coefficient $= -0.437$, $P = .002$).

3.3. Association of Twist and E-cadherin expression with the clinicopathological factors

There was a significant association between a high Twist expression and T stage (only in PGC, $P = .008$), lymph node metastasis (AGE, $P = .075$; PGC, $P = .051$), and the advanced pathological stage (AGE, $P = .019$; PGC, $P = .006$).

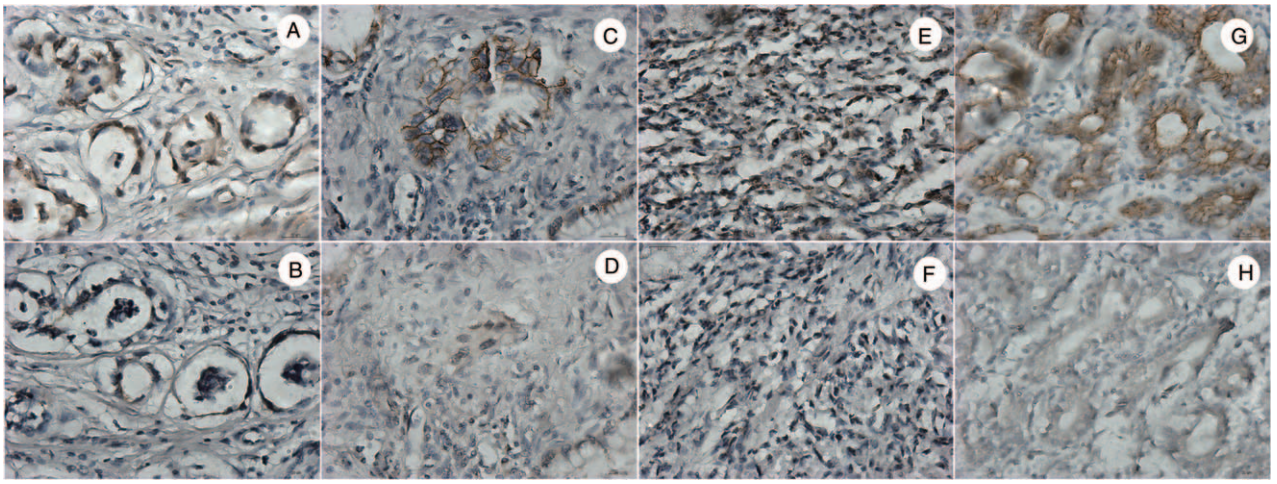

Figure 1. Sample immunohistochemical images of twist and e-cadherin staining in AGE: A, improved expression of twist; B, low expression of e-cadherin; C, high expression of e-cadherin; D, reduced expression of twist. A and B were taken from the same site of a patient. C and D were no exception. Sample immunohistochemical images of twist and e-cadherin staining in PGC: E, improved expression of twist; F, low expression of e-cadherin; G, high expression of e-cadherin; H, reduced expression of twist. E and F were taken from the same site of a patient. G and H were no exception. All the images were 400 × magnification.

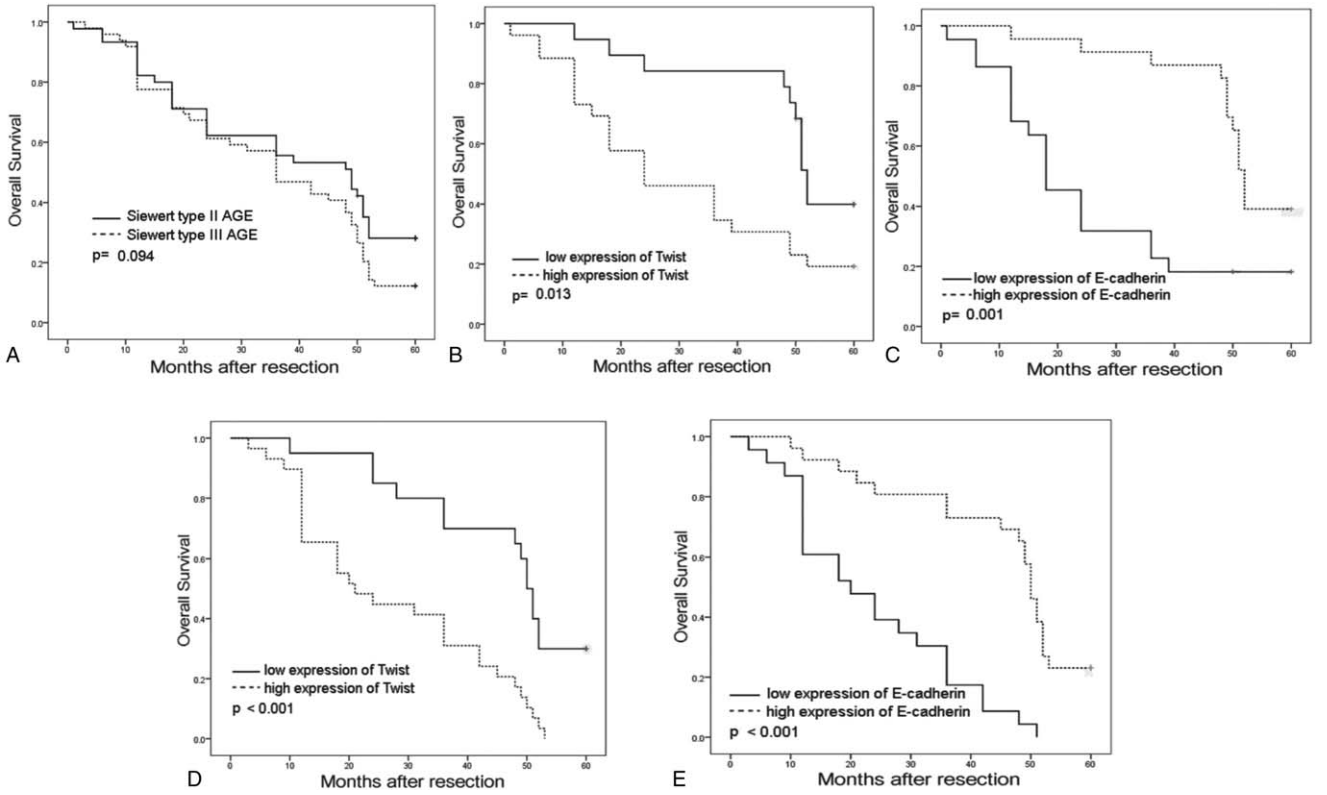

Figure 2. Kaplan–Meier estimates of overall survival according to different tumor types (A), twist expression in AGE (B), e-cadherin expression in AGE (C), twist expression in PGC (D) and e-cadherin expression in PGC (E).

A low E-cadherin expression showed similar results; however, it was not significantly associated with the advanced pathological stage of AGE ($P=.372$). In addition, a low E-cadherin expression was significantly associated with a low differentiation grade in patients with AGE ($P=.002$), while no significant association was observed in patients with PGC ($P=.425$). The correlation of Twist and E-cadherin expression with the clinicopathological factors is shown in Tables 2 and 3.

3.4. Prognostic significance of Twist and E-cadherin expression

The 5-year survival rates were 20% for the entire cohort, 28% for patients with AGE, and 12% for those with PGC; the median survival time was 42, 49.17, and 36.7 months, respectively. Figure 2 shows the OS curves. The OS was significantly shorter in patients with a high Twist expression than in those with a low Twist expression (Fig. 2B and D). In contrast, the OS was higher

Table 2

Associations of twist expression with clinicopathological parameters.

		AGE			PGC		
		High	**low**	***P* value**	**High**	**Low**	***P* value**
Gender	Male	20	17	0.435	23	13	.265
	Female	6	2		6	7	
Age	median (range)	64 (51–74)	62 (45–78)	0.604	61 (30–79)	61.5 (29–79)	.555
Smoking status	yes	13	11	0.600	4	4	.700
	no	13	8		25	16	
Drinking status	yes	13	13	0.217	16	12	.737
	no	13	6		13	8	
Differentiation	Well or moderate	11	12	0.167	8	3	.488
	Poor	15	7		21	17	
T stage	1 or 2	2	4	0.377	2	8	.009
	3 or 4	24	15		27	12	
Number of lymph node metastasis	median (range)	4 (0–19)	1 (0–13)	0.075	7 (0–72)	0 (0–21)	.051
Pathology stage	I or II	4	9	0.019	5	11	.006
	III or IV	22	10		24	9	

Table 3

Associations of e-cadherin expression with clinicopathological parameters.

		AGE			PGC		
		High	Low	*P* value	High	Low	*P* value
Gender	Male	20	17	0.459	18	18	.475
	Female	3	5		8	5	
Age	median (range)	62 (45–78)	66.5 (46–74)	0.853	63.5 (30–79)	61 (29–79)	.515
Smoking status	Yes	14	11	0.688	4	4	.856
	No	10	10		22	19	
Drinking status	Yes	13	13	0.862	14	14	.620
	No	10	9		12	9	
Differentiation	Well or moderate	17	6	0.002	7	4	.425
	Poor	6	16		19	19	
T stage	1 or 2	4	2	0.665	8	2	.080
	3 or 4	19	20		18	21	
Number of lymph node metastasis	Median (range)	1 (0–13)	5 (0–19)	0.018	0.5 (0–25)	7 (0–72)	.041
Pathology stage	I or II	8	5	0.372	12	4	.032
	III or IV	15	17		14	19	

in patients with a high E-cadherin expression than in those with a low E-cadherin expression (Fig. 2C and E). The univariate and multivariate analyses demonstrated that patients with AGE who had a high Twist expression or low E-cadherin expression showed a significantly shorter survival than those with a low Twist expression or high E-cadherin expression, respectively. Similar trends were observed for patients with PGC. The results are shown in Table 4.

4. Discussion

The TNM Classification of Malignant Tumors, 8th Edition, defines AGE as adenocarcinoma with an epicenter within 5 cm of the gastroesophageal junction and extending into the esophagus.[14] Whether there are differences between AGE and PGC in specific characteristics predictive of clinical outcomes, such as gender predilection, prognosis, and potential etiology, remains controversial.[4–6]

In the present study, our analysis revealed that smoking was a significant risk factor for AGE. PGC showed a marked association with worse differentiation grade. However, we found no significant differences in gender, age, drinking status, T stage, number of metastatic lymph nodes, M stage, or pathological stage between patients with AGE and PGC. Furthermore, no significant association was observed between the tumor site and OS.

Previously, p53 expression levels were shown to vary among the patients with AGE.[15] Twist not only affects the activity of HOXA5, an important trans-activator of p53, but also binds to the C-terminus of p53 through the Twist box, thereby hindering the key posttranslational modifications of p53 and facilitating MDM2-mediated degradation.[16] Moreover, a low E-cadherin expression showed a significant correlation with a low p53 expression.[17] To further explore the potential differences between AGE and PGC, we detected whether there were differences in Twist or E-cadherin expression in these two types of tumors. However, no significant difference was observed.

Cancer metastasis involves a complex cascade of events including detachment from the primary tumor, local invasion, entry into the circulation, extravasation, survival and proliferation at a secondary organ site, and formation of overt metastatic lesions. EMT has been implicated as a driver of metastasis and tumor invasion, during which epithelial cells lose their defining characteristics and acquire mesenchymal properties including loss of cell-cell adhesion, increased motility and invasiveness, resistance to apoptosis, and changes in cellular morphology.[18]

One of the principal characteristics of EMT is the loss of E-cadherin expression. E-cadherin participates in cell–cell adhesion and interacts with other molecules to form epithelial junctions via calcium-dependent homotypic interactions.[19] The underlying mechanisms involve mutation, epigenetic and transcriptional silencing, increased endocytosis, and proteasomal degradation. Many transcription factors, including Snail homologs and several basic helix-loop-helix factors such as Twist, are induced by the extrinsic and intrinsic stimuli during EMT. These transcription factors then bind to the E-box elements in the promoter region of E-cadherin and repress its expression. In addition, Twist also plays multiple roles in cancer initiation, progression, and metastasis; it has been shown to be involved in overriding oncogene-induced cell senescence and apoptosis,[20] increasing cancer cell resistance to chemotherapy,[21] and enhancing cancer stem cell population.[22]

Table 4

Univariate and multivariate analysis of overall survival.

	Twist						E-cadherin					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*
AGE	2.405	1.148, 5.039	.020	3.217	1.058, 9.781	.039	0.319	0.154, 0.659	.002	0.210	0.072, 0.613	.004
PGC	3.32	1.709, 6.451	<.001	2.930	1.269, 6.765	.012	0.207	0.102, 0.419	<.001	0.193	0.077, 0.482	<.001

There is little information regarding the clinical implications of Twist and E-cadherin expression in AGE. Our study revealed that the association between Twist or E-cadherin expression and clinical factors, including prognosis. The results showed that a low E-cadherin expression was significantly associated with poor differentiation and lymph node metastasis in AGE, and with T stage, lymph node metastasis, and advanced pathological stage in PGC. The expression of E-cadherin was also an independent unfavorable prognostic factor for AGE and PGC. These observations were similar to the findings of previous studies on malignancies such as lung adenocarcinoma,[23] prostate cancer,[24] gastrointestinal stromal tumors,[25] and osteosarcoma.[26]

A high Twist expression has been associated with aggressive disease, poor differentiation, metastases, advanced TNM classification, and poor clinical prognosis in hepatocellular carcinoma,[27] lung adenocarcinoma,[23] endometrial carcinoma,[28] and colorectal cancer.[29] The current study demonstrated that up-regulation of Twist had a significant association with advanced T stage, increased number of metastatic lymph nodes, and worse pathological stage in AGE and PGC, except for T stage in AGE. Similar to E-cadherin, Twist was also an independent prognostic factor for AGE and PGC.

Twist promotes EMT partly by directly repressing E-cadherin expression via recruitment of the nucleosome remodeling and deacetylase complex for gene repression and via up-regulation of Bmi1, AKT2, and YB-1, among other proteins.[30] However, Twist regulates many target proteins and E-cadherin has been shown to be modulated by several regulators. Therefore, the inhibitory effect of Twist on E-cadherin may depend on the cancer type. Increased Twist expression was shown to be associated with decreased membranous expression of E-cadherin in breast cancer, colorectal cancer, and bladder cancer[31]; however, no association was observed when E-cadherin was down-regulated in hepatocellular carcinoma.[32] The results of the current study revealed a significant inverse correlation between Twist and E-cadherin expression in patients with AGE and PGC.

The limitations of the study were the retrospective study design and the small sample size.

In conclusion, a high Twist and low E-cadherin expression were correlated with the malignant characteristics of AGE and PGC. Thus, evaluating Twist and E-cadherin expression may help to predict tumor progression and to stratify patients with AGE or PGC for efficient diagnosis and prognosis. Nevertheless, further studies are required to validate these findings.

Author contributions

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Supervision: Qing Gong.
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Visualization: Qing Gong.
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Writing – review & editing: Qing Gong.

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