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Clinical significance of ubiquilin 1 in gastric cancer

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Abstract

Ubiquilin 1 (UBQLN1) plays an essential role in the regulation of protein degradations which is involved in the pathophysiology of neurodegenerative diseases and cancer. This study aimed to investigate the expression level of UBQLN1 in gastric cancer and evaluated the relationship between its expression and clinicopathological characteristics, as well as prognostic of patients with gastric cancer. Immunohistochemistry (IHC) was used to detect the expression levels of UBQLN1 in 179 pairs of gastric cancer and adjacent normal tissues. The UBQLN1 was significantly upregulated in gastric cancer tissue. High UBQLN1 expression was associated with high histological grade, invasion, lymph node metastasis, and tumor node metastasis (TNM) stage III ($P<.001$). Multivariate Cox analysis showed that larger tumor size (HR=3.125, 95%CI: 2.031–4.808, $P<.001$), histological grade 3 (HR=15.313, 95%,CI: 8.075–29.041, $P<.001$), pT3+pT4 (HR=3.224, 95%CI: 1.389–7.483, $P=.006$), LNM (HR=4.467, 95%CI: 2.404–8.302, $P<.001$), TNM stage III (HR=2.152, 95%CI: 1.289–3.594, $P=.003$), and high UBQLN1 expression (HR=2.547, 95% CI: 1.511–4.292, $P<.001$) were significantly associated with worse prognosis of patients with gastric cancer. In conclusion, high UBQLN1 expression was an independent worse prognostic factor for patients with gastric cancer.

Abbreviations: 95% CI = 95% confidence interval, EMT = mesenchymal transformation, HR = hazard ratio, IHC = immunohistochemistry, LNM = lymph node metastasis, LVI = lymphovascular invasion, OR = odds ratio, TNM = tumor node metastasis, UBQLN1 = Ubiquilin 1, ZEB1 = zinc finger E-box binding homeobox 1.

Keywords: gastric cancer, mesenchymal transformation, prognosis, ubiquilin 1

1. Introduction

Gastric cancer is the one of most common cancers and the third leading cause of cancer-related deaths worldwide, with an approximately overall 5-year survival rate of 20%.[1,2] Like other cancers, accumulation of various epigenetic and genetic alterations is the major cause of gastric cancer.[3,4] To date, effective therapeutic strategies of gastric cancer are still limited, while its prognosis is closely related to early diagnosis.[5,6] Therefore, the identification of biomarkers for early diagnosis and prediction of prognosis of gastric cancer may help make a more personalized therapeutic schedule for patients with gastric cancer.

Ubiquilin-1 (UBQLN1), belonging to ubiquitin-like protein, plays an essential role in the regulation of protein degradation.[7] In eukaryotes, UBQLN1 links between the proteasome and ubiquitinated proteins to stimulate ubiquitinated protein degradation, facilitate misfolded autophagy regulation, and protein degradation.[8,9] Disruption of UBQLN1 function is involved in pathological process of a variety of human neurodegenerative disorders, such as Alzheimer's disease[10,11] and Huntington's disease.[12] Furthermore, recent studies have shown that UBQLN1 regulates the development and progression of human cancer.[13–15] For example, zinc finger E-box binding homeobox 1 (ZEB1) is a mesenchymal transformation (EMT) activator, which plays a crucial role in cancer progression and metastasis. UBQLN1 mediates EMT through inhibiting ZEB1 expression,[14,15] and thus regulates migration and invasion of cancer cells.[14,15] In addition, UBQLN1 is upregulated in breast cancer and knockdown of UBQLN1 enhances radiosensitivity of breast cancer cells. UBQLN1 overexpression is an independent poor prognostic factor for patients with breast cancer.[16] However, Yang et al[13] found that UBQLN1 was regulated by miR-155 and was downregulated in radioresistant nasopharyngeal carcinoma. UBQLN1 may play different roles in different types of cancer. These results indicate that UBQLN1 plays an important role in human cancer. However, the role of UBQLN1 in gastric cancer remains largely unknown. In the present study,

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ZL and HY contributed equally to this work.

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we investigated UBQLN1 expression in gastric cancer tissues and explored whether its expression was related to the clinicopathological features and prognosis of patients with gastric cancer.

2. Materials and methods

2.1. Patients

Patients with histologically confirmed gastric adenocarcinoma were recruited who received surgical resection of gastric cancer between January 2010 and December 2011 at Taizhou People's Hospital. Prior to surgery, patients did not received any anticancer treatment. The exclusion criteria were as follows: history of other cancer; cancer of unknown primary origin, and history of previous cancer treatment. All patients were followed for up to 5 years until they died. Histological classifications were assigned according to Lauren's classification. Tissues were formalin-fixed, conventionally dehydrated, and paraffin embedded. All patients provided informed written consent according to the protocol approved by the Ethic Committee of Taizhou People's Hospital.

2.2. Immunohistochemistry (IHC)

The UBQLN1 expression was measured by IHC in 179 pairs of gastric cancer and paracancerous tissues using UBQLN1 antibody (DAKO, CA) at a dilution of 1:200. Experimental procedures were described as previous study.[17] The tumor lymphovascular invasion (LVI) was evaluated by D2-40 (at a dilution of 1:50, DAKO, CA). The IHC slides were blindly reviewed by 2 independent pathologists. The presence of LVI was considered positive only when at least 1 neoplastic cell embolus was clearly visualized inside a D2-40 positive lymph vessel. The UBQLN1 expression was evaluated using the semi-quantitative method described as previous study.[16] In brief, the staining intensity was graded as 0 (negative), 1 (weak, 2 (moderate), and 3 (strong), and the percentage of staining cells was scored as 0 (no staining), 1 (1–10%), 2 (11–50%), 3 (51–80%), and 4 (81–100%). The total score (staining intensity × the percentage of staining cells) < 4 were defined as low expression, whereas score ≥4 were defined as high expression.

2.3. Statistical analyses

All statistical data were performed by SPSS 20.0 statistical software (IBM SPSS, CA). The relationship between UBQLN1 expression and clinical variables was analyzed by χ^2 test and Fisher's exact test. The survival analysis was performed by Kaplan–Meier method and the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated by Cox's proportional hazards model. A $P < .05$ was considered statistically significant.

3. Results

3.1. Clinicopathological characteristics of patients with gastric cancer

A total of 179 patients were included in this study. Clinical characteristics of patients were described in Table 1. Briefly, the median age of the patients was 66.0 years (range: 40.0–80.0). Of all the patients, 133 cases (74.3%) were males. Ninety-three patients (52.0%) presented with lymph node metastasis (LNM) at the time of diagnosis. Eighty-eight cases were poor differentiation. In addition, 62 cases were stage I (34.6%), 54 cases were stage II (30.2%), and 63 cases were stage III (35.2%).

Table 1

Clinical characteristics of patients with gastric cancer.

General characteristics	All (n = 179)
Age at primary diagnosis, years	65.0 ± 8.3
Male, n (%)	133 (74.3)
Diameter of the tumor, cm	Median: 4.0 (0.8–12.5)
Follow-up period, month	Median: 42.2 (39.4–45.0)
Histological grade	
1+2	91 (50.8)
3	88 (49.2)
Location	
Cardia	91 (50.8)
Antrum	46 (23.5)
Body	42 (23.5)
LVI	
Negative	136 (76.0)
Positive	43 (24.0)
Lymph node status	
Negative	86 (48.0)
Positive	93 (52.0)
pT category	
pT1	47 (26.3)
pT2	19 (10.6)
pT3	20 (11.2)
pT4	93 (52.0)
TNM stage	
I	62 (34.6)
II	54 (30.2)
III	63 (35.2)

LVI = lymphovascular invasion, TNM = tumor node metastasis.

3.2. Association between UBQLN1 expression and clinical factors

UBQLN1 was mainly expressed in the cytoplasm of gastric cancer cells (Fig. 1). Positive expression rate of UBQLN1 in gastric cancer tissues was 69.3% (124/179), whereas the paracancerous tissues were negative for UBQLN1. The expression levels of UBQLN1 in gastric cancer tissues were significantly higher than those in noncancerous tissues ($P < .001$).

We further examined the association between UBQLN1 expression and clinical features of patients with gastric cancer. Statistical analysis revealed that UBQLN1 expression was significantly related to histological grade, LVI, LNM, pT category, and TNM stage ($P < .05$, Table 2). There was also a borderline association between UBQLN1 expression and tumor size ($P = .053$). However, there was no difference between UBQLN1 expression and age, sex, and tumor location ($P > .05$).

3.3. Associations between UBQLN1 expression and patient survival

Seventy-seven patients died during the follow-up period. The median survival time (MST) was 42.2 months (95% CI 39.437–44.965). Patients with high UBQLN1 expression had shorter survival time than those with low UBQLN1 expression ($P = .010$, Fig. 2). In univariate analysis, larger tumor size (HR = 3.063, 95%CI: 2.046–4.585, $P < .001$), histological grade 3 (HR = 7.29, 95%CI: 4.541–11.704, $P < .001$), pT3 + pT4 (HR = 10.239, 95% CI: 5.288–19.826, $P < .001$), LVI (HR = 1.843, 95%CI: 1.204–

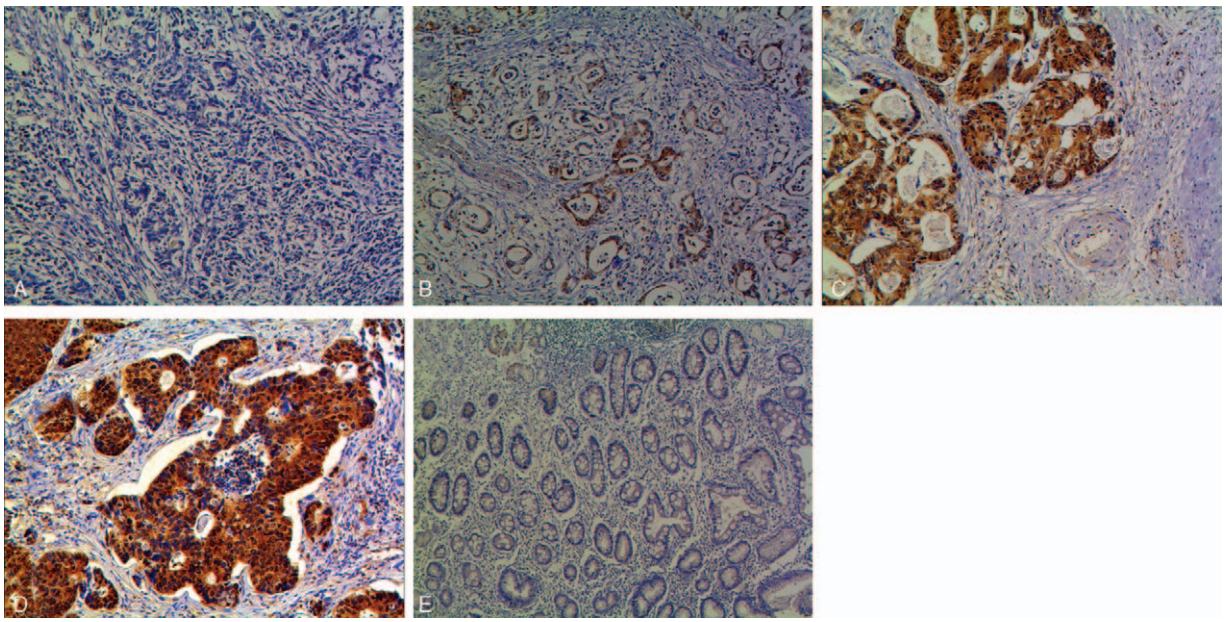

Figure 1. IHC staining of UBQLN1 in gastric cancer and paracancerous tissues (200×). UBQLN1 was significantly upregulated in gastric cancer tissues ($P < .001$). (A–D), gastric cancer with negative (A), weak (B), moderate (C), and strong (D) UBQLN1 expression. (E) UBQLN1 staining was negative in paracancerous tissues. IHC = immunohistochemistry, UBQLN1 = Ubiquilin 1.

Table 2

Relationship between UBQLN1 expression and clinicopathological characteristics.

Variables	UBQLN1 expression		*P* value
	High	Low	
Age, years			
<65	61	22	.330
≥65	63	33	
Sex			
Male	91	42	.715
Female	33	13	
Tumor size, cm			
≤5	80	44	.053
>5	44	11	
Histological grade			
3	79	12	<.001
1+2	45	43	
Location			
Cardia	69	22	.121
Antrum	30	16	
Body	25	17	
LVI			
Negative	90	46	.131
Positive	34	9	
LNM			
Yes	77	15	<.001
No	47	40	
pT category			
pT1+pT2	35	31	<.001
pT3+pT4	89	24	
TNM stage			
I	31	31	<.001
III	57	6	

LNM = lymph node metastasis, LVI = lymphovascular invasion, UBQLN1 = Ubiquilin 1.

2.818, $P = .005$), LNM (HR = 6.890, 95%CI: 4.238–11.202, $P < .001$), TNM stage III (HR = 7.273, 95%CI: 4.728–11.189, $P < .001$), high UBQLN1 expression (HR = 1.814, 95% CI: 1.144–2.877, $P = .011$) were significantly associated with poor prognosis of patients with gastric cancer (Table 3). Multivariate Cox analysis demonstrated that larger tumor size (HR = 3.125, 95%CI: 2.031–4.808, $P < .001$), histological grade 3 (HR = 15.313, 95%,CI: 8.075–29.041, $P < .001$), pT3 + pT4 (HR = 3.224, 95%CI: 1.389–7.483, $P = .006$), LNM (HR = 4.467, 95% CI: 2.404–8.302, $P < .001$), TNM stage III (HR = 2.152, 95%CI: 1.289–3.594, $P = .003$), and high UBQLN1 expression (HR = 2.547, 95%CI: 1.511–4.292, $P < .001$) were independent worse prognostic factors for patients with gastric cancer (Table 3).

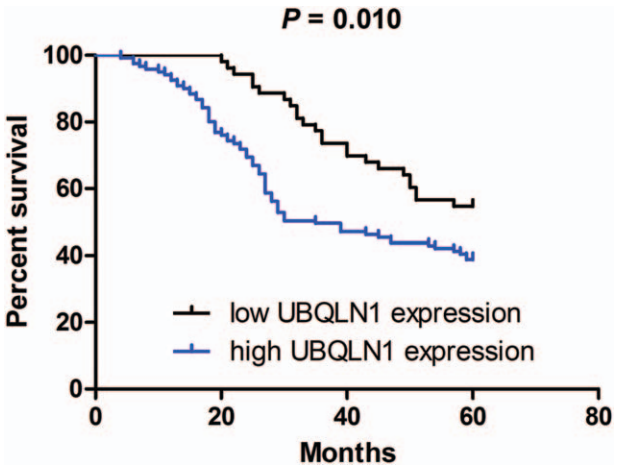

Figure 2. Kaplan–Meier curves according to UBQLN1 expression in patients with gastric cancer. Patients with high UBQLN1 expression had a shorter survival time than those with low UBQLN1 expression ($P = .010$). UBQLN1 = Ubiquilin 1.

Table 3

Univariate and multivariate Cox regression analysis of overall survival in patients with gastric cancer.

	Univariate analysis		Multivariate analysis	
Features	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Age (years), < 65 vs ≥ 65	0.886 (0.596–1.316)	.548		
Sex, male vs female	1.099 (0.702–1.722)	.679		
Tumor size (cm), > 5 vs ≤ 5	3.063 (2.046–4.585)	<.001	3.125 (2.031–4.808)	<.001
Histological grade, 3 vs 1+2	7.29 (4.541–11.704)	<.001	15.313 (8.075–29.041)	<.001
Location, cardia vs antrum+body	1.126 (0.923–1.374)	.240		
pT category, pT3+pT4 vs pT1+pT2	10.239 (5.288–19.826)	<.001	3.224 (1.389–7.483)	.006
LVI, positive vs negative	1.843 (1.204–2.818)	.005	1.506 (0.948–2.391)	.083
LNM, positive vs negative	6.890 (4.238–11.202)	<.001	4.467 (2.404–8.302)	<.001
TNM stage, III vs I+II	7.273 (4.728–11.189)	<.001	2.152 (1.289–3.594)	.003
UBQLNl expression, high vs low	1.814 (1.144–2.877)	.011	2.547 (1.511–4.292)	<.001

HR = hazard ratio, LNM = lymph node metastasis, LVI = lymphovascular invasion, UBQLN1 = Ubiquilin 1.

4. Discussion

In the present study, it was demonstrated that high UBQLN1 expression was closely related to high histological grade (grade 3), TNM stage III, LNM, and poor prognosis of patients with gastric cancer. UBQLN1 may serve as a biomarker for identifying patients at high risk of mortality who might require other treatment strategies.

The N-terminal ubiquitin-like domain of UBQLN1 mediates interaction with the proteasome, whereas its C-terminal ubiquitin-associated domain preferentially binds poly-ubiquitinated proteins. However, UBQLN1 is not able to be covalently attached to target proteins, which is different from those small ubiquitin-like proteins, such as NEDD8.[18] It has been suggested that UBQLN1 acts as an adaptor protein/ubiquitin shuttle factor mediate protein degradation through the ubiquitin proteasome system and the autophagy–lysosomal system, and protects cells from oxidative stress.[19–21] Therefore, UBQLN1 plays an important role in a variety of cellular processes and is involved in the pathophysiology of neurodegenerative diseases and cancer.[22,23]

UBQLN1 is frequently overexpressed in breast[16] and lung cancers.[23,24] However, Shah et al[14] reported that UBQLN1 was downregulated in lung cancer, which remained uncertainty in data accuracy due to lack of validation. UBQLN1 overexpression promotes autophagosome formation and confers radioresistance to breast cancer cells.[20] A study by Yang et al[13] showed a different result that downregulation of UBQLN1 was associated with radioresistance of nasopharyngeal carcinoma cells. Furthermore, UBQLN1 regulates apoptosis through interaction with BCLb and thus stabilizing BCLb protein.[23] Elevated levels of UBQLN1 is associated with poor prognosis of patients with breast and lung cancers.[16,23] In the present study, we found that high UBQLN1 expression contributed to malignant behavior and poor prognosis of gastric cancer. Inhibition of UBQLN1 reduced cell viability and suppresses autophagy in breast cancer cells. A study by Shah et al[14] revealed that UBQLN1 inhibited lung adenocarcinoma cells migration by repressing the expression of mesenchymal markers including Vimentin, Snail, and ZEB1. Wang et al[15] found that UBQLN1 expression was affected by miR-675-5p in pancreatic cancer, and regulated the protein level of ZEB1. UBQLN1 regulates migration and invasion of different types of cancer cells through inhibiting ZEB1 expression which is required for induction of mesenchymal-like properties.[14,15] Since UBQLN1 overexpression was significantly associated with pT category, LNM, and TNM stage, elevated UBQLN1 expression may promotes gastric cancer cells invasion and metastasis. Therefore, gastric cancer with UBQLN1 overexpression has tendency to exhibit considerably more malignant behavior such as poor differentiation and metastasis. Given that UBQLN1 overexpression confers a poor prognosis in gastric cancer patients, in addition to more malignant behavior, gastric cancer with UBQLN1 overexpression may likely develop resistance to anticancer drugs. Patients with UBQLN1 overexpression might not benefit from anticancer drug treatment, and thus be closely monitored for response to therapy and receive further treatment. Our findings revealed a potential oncogenic role for UBQLN in gastric cancer. Since the sample size is relatively small, further well characterized large-scale studies are required to elucidate the exact role of UBQLN in gastric cancer.

In summary, our findings revealed that UBQLN1 was upregulated in gastric cancer and high UBQLN1 expression was associated with malignant behavior of gastric cancer. High UBQLN1 expression was an independent worse prognostic factor for patients with gastric cancer.

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