Original paper

Clinicopathological significance of Obg-like ATPase 1 and its association with Snail in gastric cancer

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Obg-like ATPase 1 (OLA1) is a member of the Obg family of P-loop NTPases and has recently been detected in several human cancer cells. However, its expression type and clinical relevance in gastric cancer remains unclear.

In the present study, mRNA level of OLA1 in gastric cancer (GC) was detected in 2 datasets downloaded from the open Gene Expression Omnibus database and 30 cancer tissues. Immunohistochemistry was performed on GC and its association with Snail in 334 GC patients.

The results showed that OLA1 mRNA and protein were elevated in GC tissues. High expression of OLA1 was significantly associated with aggressive features, such as tumour size, lymph-node-metastasis and tumour-nodus-metastases stage ($p = 0.0146, p = 0.0037, p < 0.001$, respectively).

Moreover, high levels of OLA1 predicted worse overall survival. Multivariate Cox regression analysis indicated that high expression of OLA1 was an independent prognostic factor for poor overall survival ($p = 0.009$). Additionally, OLA1 expression was positively correlated with Snail, and a combination of them revealed improved prognostic accuracy for GC patients. High expression of OLA1 predicts poor prognosis in GC patients and may be serviced as a novel target for GC.

Key words: OLA1, gastric cancer, prognosis, immunohistochemistry, Snail.

Introduction

Gastric cancer (GC) is the second most common cancer and the leading cause of cancer-related deaths in China [1]. Although the incidence and mortality rates of GC have decreased significantly in recent years because of improvements in surgical techniques and adjuvant chemotherapy, the prognosis remains poor [2]. Therefore, understanding the molecular mechanisms of the pathogenesis of GC is indispensable for improving diagnosis, prognosis, and treatment of this form of cancer.

Obg-like ATPase 1 (OLA1) is a member of the translation-factor-related class, Obg family, and YchF subfamily of P-loop GTPases [3, 4]. Previous studies found that OLA1 plays multiple roles in the regulation of cell proliferation and cell survival through acting as an intrinsic regulator in cellular stress responses such as oxidative stress and heat shock [5, 6]. Moreover, OLA1 is overexpressed in multiple human malignancies including colon, rectum, stomach, ovary, lung, and uterus [7]. Accumulating evidence indicates that OLA1 participates in tumourigenesis and progression. In breast cancer, knockdown of OLA1 inhibits cellular motility and invasion [8]. In lung cancer, OLA1 contributes to cell epithelial-mesenchymal transition (EMT), and its overexpression has been associated with clinical progression and poor survival [9]. Conversely, in oral cancer, Liu *et al*. reported that OLA1 inactivated the EMT process and inhibited can-

cer cell metastasis [10]. The role of OLA1 in different cancers remains debatable. However, the expression pattern and clinical significance of OLA1 in GC have not yet been elucidated.

In this study, we investigated OLA1 expression and characterized its clinicopathological significance in a large cohort of GC tissues. We found that OLA1 expression increased in both mRNA and protein level in GC tumour tissues. OLA1 overexpression is associated with aggressive clinicopathological features and predicts an unfavourable prognosis. Additionally, OLA1 expression is positively related to Snail, an EMT key regulator, indicating a potential role of OLA1 in the EMT process of GC cells. An earlier version of this study was presented as PREPRINT in Research Square [11].

Material and methods

Gene expression profile data

Gene expression profile data of GC (GSE118916 and GSE79973) were downloaded from the open Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). Bioinformatic software Qlucore Omics Explorer (QOE 3.1) (http://www.qlucore.com/) was used to analyse the expression of OLA1 mRNA as described previously [12].

Tissue microarray and patient clinical information

Paraffin-embedded pathological specimens (30 GC) and paired adjacent non-tumour tissues were collected to validate the OLA1 mRNA level through quantitative reverse transcription polymerase chain reaction (qRT-PCR). Simultaneously, 334 formalin-fixed, paraffin-embedded GC tumour specimens and 30 matched adjacent non-tumour tissues were gathered to construct tissue microarray (TMA) according to the method described previously, and they were employed to check the OLA1 protein expression by immunohistochemical staining. All samples in this study were collected from the First Affiliated Hospital of Sun Yat-sen University, with a distinctive pathological diagnosis of gastric adenocarcinoma. No patients received immunotherapy, chemotherapy, or radiotherapy before their surgical operation. All the samples were collected with the patient's informed consent after approval from the Institute Research Medical Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and Nanfang Hospital of Southern Medical University.

RNA extraction and quantitative reverse transcription polymerase chain reaction

Total RNA was isolated from 30 paraffin-embedded GC tumour tissues and matched adjacent non-tumour tissues using TRIzol reagent (Invitrogen, USA). The OLA1 mRNA level was determined by qRT-PCR using a relative quantification method by normalizing to GAPDH. The primers used are as follows: OLA1, forward primer (5'-TGGACAAGTATGACCCAGGT-3'); reverse primer (5'-GCTGCAAACCCAGCCTTAATG-3') and GAPDH, forward primer (5'-TGCACCACCAACTGCTTAGC-3'; reverse primer (5'-GGCATGGACTGTGGTCATGAG-3').

Immunohistochemical staining and evaluation

Immunohistochemical staining was performed on TMA sections using a standard streptavidin-biotin-peroxidase complex method as previously described [12]. Expression of OLA1 and Snail protein were determined semi-quantitatively by combining the proportion and intensity of the positively stained tumour cells. Brown staining in cytoplasm was defined as positive staining of OLA1 protein, while nuclear immunoreactivity for Snail protein was classified as positive signal. The percentage of positively stained tumour cells was scored as follows: 0 (no positive tumour cells); 1 (1–25% positive tumour cells); 2 (26–50% positive tumour cells); 3 (51–75% positive tumour cells); and 4 (76–100% positive tumour cells). Staining intensity was scored as follows: 0 (no staining); 1 (weak staining); 2 (moderate staining); and 3 (strong staining). The staining index was calculated as the staining intensity score multiplied by the proportion of positively stained tumour cells (values 0–12). Samples with a summed score of less than 4 were defined as exhibiting low expression, while those with a summed score of 6–12 were defined as exhibiting high expression.

Statistical analysis

Statistical analysis was performed with the SPSS package (version 20.0). Student's *t*-test was used to compare OLA1 expression between tumour tissues and adjacent non-tumour tissues. Pearson χ^2 tests were performed to determine the correlation between OLA1 protein expression and clinicopathologic parameters. Kaplan-Meier analysis was used for univariate survival analysis, and the log-rank test was applied to compare different survival curves. The multivariate Cox regression model was used to evaluate the potential independent prognostic factors and 95% confidence intervals of the hazard ratio (HR). For all analyses, $p < 0.05$ was regarded as statistically significant.

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Ethical approval

Owing to the retrospective nature of the study, the Ethics Review Committee of the Affiliated Hospital of Sun Yat-sen University gave written exemption.

Data availability

All data are accessible upon reasonable request to the corresponding author.

Results

OLA1 is highly expressed in gastric cancer tissues

To detect the expression level of OLA1 mRNA in GC, we first downloaded 2 cohorts of GC datasets (GSE118916, GSE79973) from the GEO database. Bioinformatic software QOE3.1 was employed to analyse the expression level of OLA1 mRNA in GC tissues and adjacent non-tumour tissues. As shown in Figure 1A, the OLA1 mRNA level was significantly higher in GC tissues compared with that in adjacent non-tumour tissues ($p < 0.01$, $p < 0.05$, respectively) (Fig. 1A). Then, qRT-PCR assay was used to confirm these results in 30 GC tissues and paired adjacent non-tumour tissues. Our results showed that OLA1 mRNA expression was significantly higher in

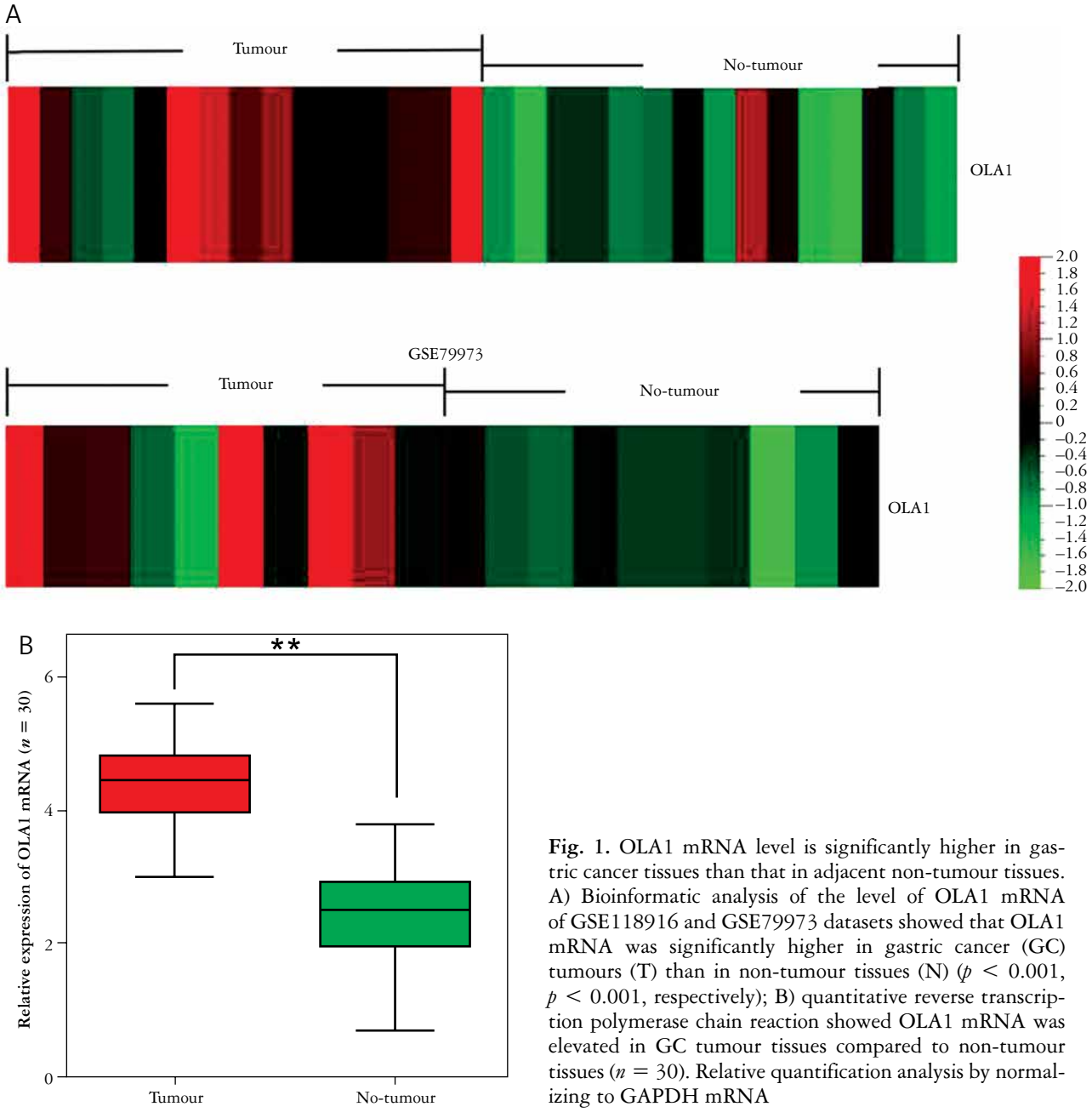


Fig. 1. OLA1 mRNA level is significantly higher in gastric cancer tissues than that in adjacent non-tumour tissues. A) Bioinformatic analysis of the level of OLA1 mRNA of GSE118916 and GSE79973 datasets showed that OLA1 mRNA was significantly higher in gastric cancer (GC) tumours (T) than in non-tumour tissues (N) ($p < 0.001$, $p < 0.001$, respectively); B) quantitative reverse transcription polymerase chain reaction showed OLA1 mRNA was elevated in GC tumour tissues compared to non-tumour tissues ($n = 30$). Relative quantification analysis by normalizing to GAPDH mRNA

GC tissues than that in adjacent non-tumour tissues ($p < 0.01$) (Fig. 1B).

To further evaluate the expression pattern of OLA1 protein in GC tissues, immunohistochemical staining was conducted in GC TMA including 334 GC tissues and 30 corresponding non-tumour tissues. OLA1 protein was detected in both GC tissues and adjacent non-tumour tissues. High expression of OLA1 protein was detected in 81.1% (252/334) of primary GC tissues compared with 33.3% (10/30) of adjacent non-tumour tissues ($p < 0.01$) (Fig. 2A–C). These results suggest that OLA1 was highly expressed in GC tissues at both the mRNA and protein level.

Association between OLA1 protein expression and clinicopathological parameters of gastric cancer

To detect the clinical significance of OLA1 in GC, the relationships between the expression of OLA1 and clinicopathological parameters were analysed. High or low expression rates of OLA1 protein in GC with respect to several standard clinicopathological features are presented in Table I. It was shown that OLA1 protein overexpression was significantly related to tumour size, lymph-node-metastasis (LNM), tumour-node-metastases (TNM) stage. The frequency of OLA1 positivity was significantly higher in patients with a tumour size ≥ 5 cm compared with patients with a tumour size < 5 cm (81.9%, 122/149 vs. 70.3%, 130/185, $p = 0.0146$). Furthermore, the expression of OLA1 was higher in GC specimens with LNM compared with those without LNM (79.9%, 187/234 vs. 65.0%, 65/100, $p = 0.0037$). The proportion of OLA1-positive samples was lower in early TNM stage (I + II) than those in advanced stage (III + IV) (52.9%, 62/117 vs. 87.6%, 190/217, $p < 0.001$). There was no significant correlation between OLA1 protein expression and the other clinicopathological parameters, such as gender, age at surgery, and histological type.

OLA1 predicts inferior prognosis in gastric cancer patients

Based on the immunohistochemical staining results, the association of OLA1 protein expression and survival time was detected. Kaplan-Meier survival analysis showed that the mean survival time of patients with high expression of OLA1 in GC tissues was 31.34 ±1.73 months, which was significantly shorter compared with patients in the low OLA1 expression group, at 42.54 ±2.95 months ($p = 0.002$) (Fig. 2D). Then, multivariate Cox regression analysis showed that high expression of OLA1 was an independent inferior prognostic factor for GC patients (hazard ratio, 0.573; 95% confidence interval, 0.376–0.872; $p = 0.009$), as well as other clinicopathological variables (LNM and TNM stage) (Table II).

OLA1 expression is positively correlated with Snail in gastric cancer tissues

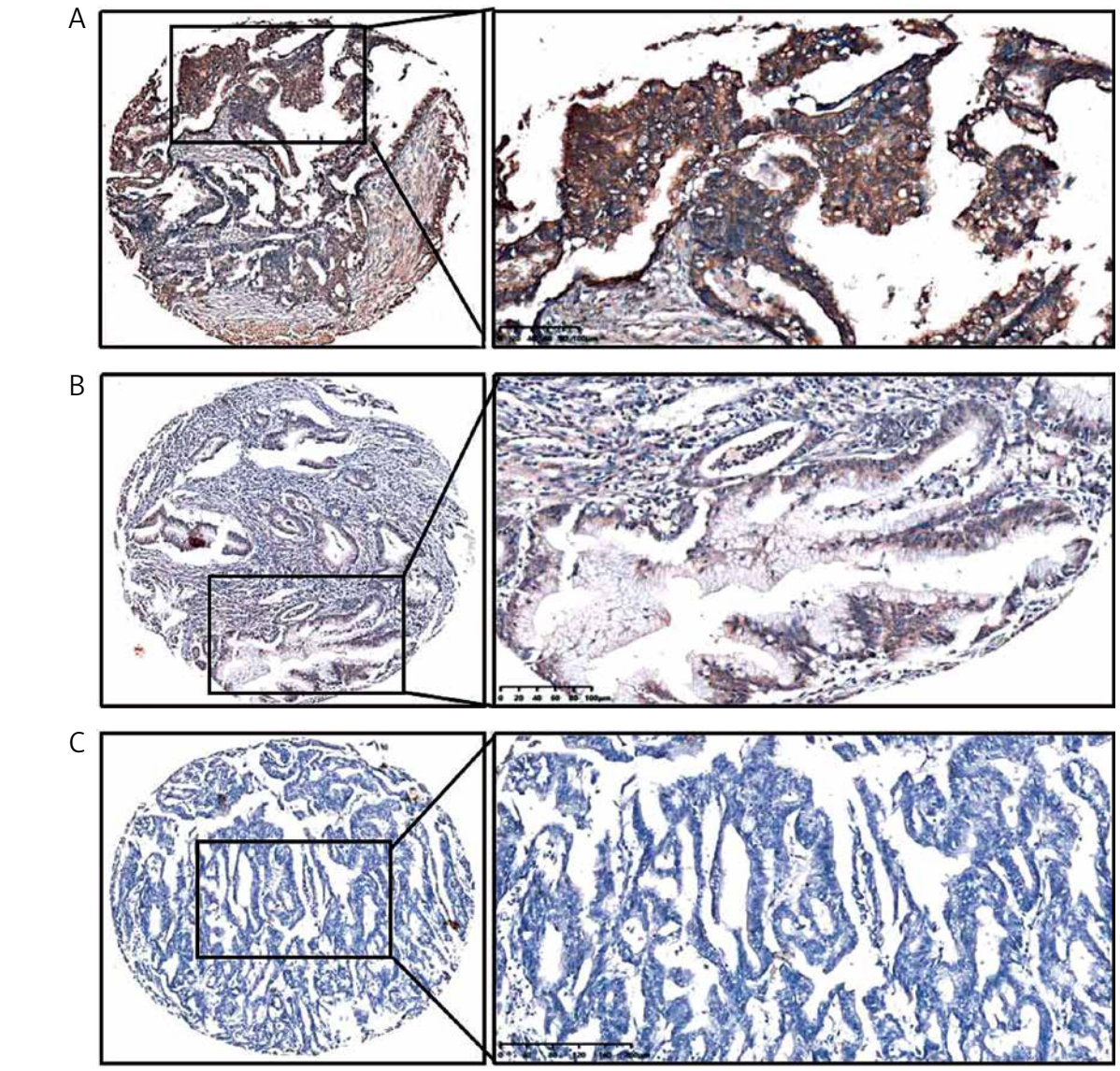
A previous study reported that OLA1 contributed to EMT in lung cancer by modulating Snail [9], which is a main EMT activating transcriptional factor. Upregulation of Snail plays a vital role in the initiation and development of GC [13]. Thus, we aimed to reveal the relationship between OLA1 and Snail expression in GC by immunohistochemical staining. We found that high Snail expression was detected in 65.6% (219/334) of GC tissues. Furthermore, Spearman's rank correlation analysis indicated a significantly positive correlation between expression of OLA1 and Snail in GC tissues ($r = 0.334$, $p < 0.001$) (Fig. 3A, B, Table III).

Combining OLA1 expression with Snail reveals improved prognostic accuracy for gastric cancer patients

To analyse the prognostic value of combining OLA1 and Snail in GC, we divided the patients into 4 groups: OLA1 high expression/Snail high expression (OLA+/Snail+), OLA1 high expression/Snail low expression (OLA1+/Snail–), OLA1 low expression/Snail high expression (OLA1–/Snail+), and OLA1 low expression/Snail low expression (OLA1–/Snail–). The results revealed that the OLA1+/Snail+ group had the worst prognosis, while the most favourable prognosis was seen in the OLA1–/Snail– group ($p < 0.001$) (Fig. 3C). These data showed that the combination of OLA1 elevation and Snail increase in GC tissues seems to be predictive of the poorest prognosis.

Discussion

OLA1, belongs to the Obg family of P-loop NTPases. It is required for normal progression of mammalian development [14] and may serve as a "molecular switch" regulating multiple cellular processes [15]. Nevertheless, the role of OLA1 in cancer progression remains poorly understood. In the present study, we assessed the expression pattern of OLA1 mRNA and protein in GC tissues and explored the clinical significance of OLA1 protein in a large cohort of GC patients. The results showed that OLA1 expression markedly increased in GC tissues compared with the adjacent non-tumour tissues both in mRNA and protein level, and overexpression of OLA1 protein was significantly associated with aggressive clinicopathological features, such as tumour size, LNM, and advanced TNM stage. Importantly, OLA1 overexpression predicted unfavourable prognosis and may be a potential independent prognostic marker of GC patients. Moreover, OLA1 is closely positively related to Snail1, and the combination of these 2 markers reveals improved prognostic accuracy for GC patients.



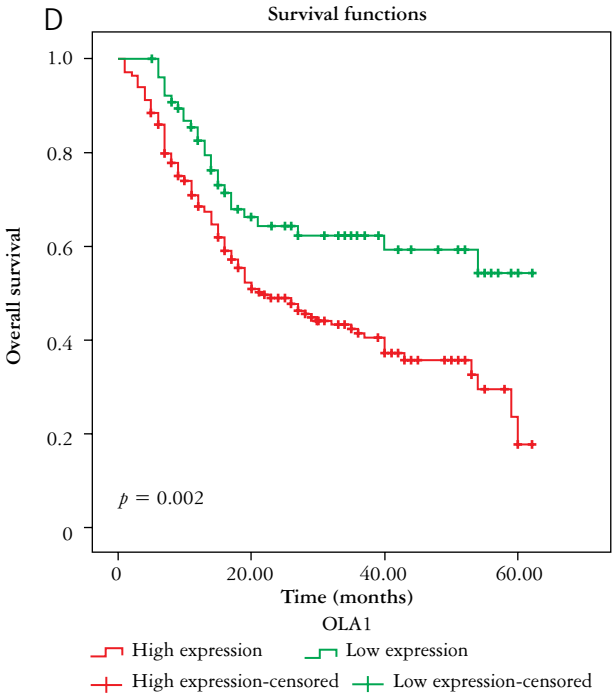


Fig. 2. OLA1 protein is highly expressed in gastric cancer tissues and predicts poor prognosis. A) High expression of OLA1 protein in the cytoplasm of gastric cancer (GC) cells (staining index, $4 \times 3 = 12$); B) moderate expression of OLA1 protein in the cytoplasm of GC cells (staining index, $4 \times 2 = 8$); C) low expression of OLA1 protein in the cytoplasm of GC cells (staining index, $4 \times 0 = 0$), the right panel indicates higher magnification with representative zone (400×); D) Kaplan-Meier analysis of overall survival for GC patients showed that GC patients with high OLA1 expression had a significantly shorter overall survival time than those with low expression of OLA1 ($p = 0.002$)

Table I. Relation between OLA1 protein expression and clinicopathological

Parameters		OLA1 protein expression		χ^2	*p*-value
		High expression (%)	Low expression (%)		
Gender					
Male	233	176 (75.5)	57 (24.5)	0.003	0.9551
Female	101	76 (75.2)	25 (24.8)		
Age					
< 57[c]	172	124 ((72.1)	48 (27.9)	2.156	0.1420
≥ 57	162	128 (79.0)	34 (31.0)		
Tumour size					
≥ 5 cm	149	122 (81.9)	27 (19.1)	6.004	0.0146
< 5 cm	185	130 (70.3)	55 (29.7)		
Histological type					
Intestinal	265	204 (76.9)	61 (23.1)	1.625	0.2023
Diffuse	69	48 (69.6)	21 (30.4)		
Lymph node metastasis					
Present	234	187 (79.9)	47 (20.1)	8.413	0.0037
Absent	100	65 (65.0)	35 (35.0)		
TNM stage					
I + II	117	62 (52.9)	55 (47.1)	49.03	< 0.0001
III + IV	217	190 (87.6)	27 (12.4)		

Table II. Multivariate analysis on overall survival (Cox regression model)

Parameters	Hazard ratio	95% confidence interval	*p*-value
Lymph node metastasis[a]	0.296	0.145–0.605	0.001
TNM stage[b]	1.888	1.363–2.614	0.000
OLA1 expression[c]	0.573	0.376–0.872	0.009

[a] – present vs. absent, [b] – stage I + II vs. stage III + IV, [c] – high expression vs. low expression

Emerging evidence has demonstrated the expression type and clinical implications of OLA1 in multiple malignancies. Sun *et al*. [7] indicated that OLA1 (DOC45) mRNA was overexpressed in cancers of the colon, rectum, stomach, ovary, uterus, and lung, and the elevated expression of OLA1 protein was confirmed in colon cancer tissues. Consistent with our results, Huang *et al*. [16] reported that OLA1 was highly expressed in hepatocellular carcinoma, and correlated with unfavourable clinical characteristics like tumour size, portal vein tumour thrombus, and TNM stage, and predicted poor survival. Bai *et al*. [9] found that OLA1 expression was significantly higher in lung cancer tissues than that in paired normal lung tissues. Moreover, OLA1 expression was positively related with LNM and TNM stage, increased expression of OLA1 mRNA was significantly associated with shorter overall survival in lung cancer patients. However, the same team previously found that OLA1 expression was not significantly correlated with clinical parameters including tumour invasion stage and LNM in breast cancer. Furthermore, lower OLA1 protein expression was associated with higher risk of relapse and a decreased disease-specific survival in breast cancer patients [17]. These distinctive results may indicate that the clinical significance of OLA1 relied on different cancer types.

With regard to the biological role of OLA1 in cancer cells, it is also still controversial. In hepatocellular carcinoma, downregulation of OLA1 significantly inhibited the proliferation, migration, invasion, and tumourigenicity of hepatocellular carcinoma cells [16]. Conversely, a recent study on oral carcinoma revealed that knockdown OLA1 enhanced cell migrative ability but had no effect on cell proliferation [10]. Even in the same type of cancer, the role of OLA1 needs to be further explored. For example, lower expression of OLA1 abrogated cell motility and invasion

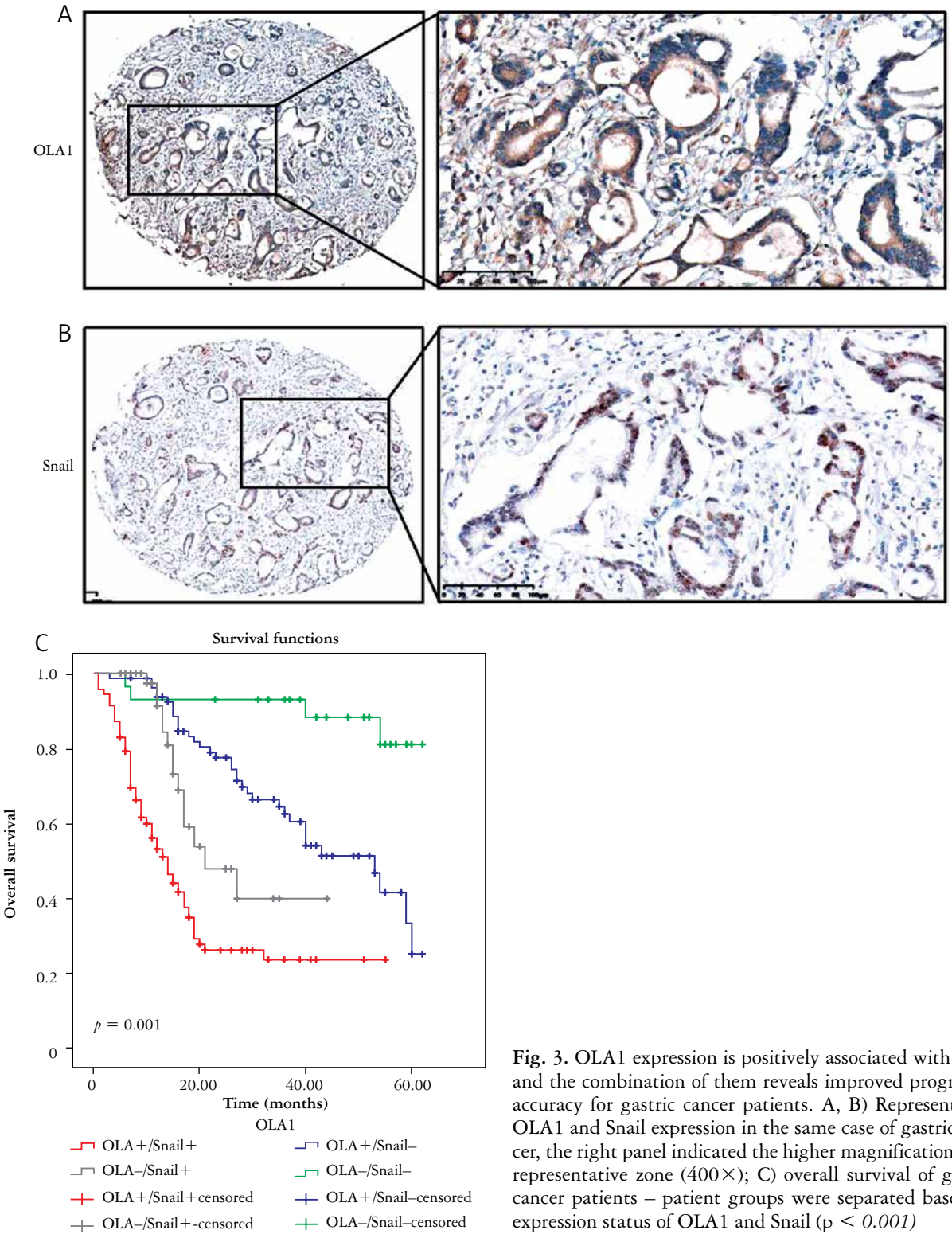


Fig. 3. OLA1 expression is positively associated with Snail and the combination of them reveals improved prognostic accuracy for gastric cancer patients. A, B) Representative OLA1 and Snail expression in the same case of gastric cancer, the right panel indicated the higher magnification with representative zone (400×); C) overall survival of gastric cancer patients – patient groups were separated based on expression status of OLA1 and Snail ($p < 0.001$)

Table III. Correlation between OLA1 and Snail expression in GC tissues

Parameters		All Cases	Snail expression		*R*	*P*-value
			High	Low		
OLA1 expression	High (%)	252	188 (74.6)	64 (25.4)	0.334	< 0.001
	Low (%)	82	31 (37.8)	51 (62.2)		

in vitro [8], while another report from the same group reported that downregulation of OLA1 has no or a negative impact on cell growth in vitro but promotes tumour growth *in vivo* [17]. Similar to this, knockdown of OLA1 negatively affected cell proliferation in colorectal cancer cells *in vitro* [7] but increased tumour growth in animal models [18]. These conflicting findings reflect the complicated role of OLA1 in tumourigenesis and tumour progression. When we further detect the functional role of OLA1 in GC, more comprehensive experiments should be employed.

Previous studies reported that OLA1 was closely related to EMT, which was the key step of tumour metastasis [19]. Bai *et al*. [9] found that OLA1 contribute to EMT by modulating GSK3β/Snail/E-Cadherin pathway in lung cancer. In breast cancer, escalated expression of OLA1 promoted the EMT process in tumour cells through TGF-β/Smad signalling cascades, resulting in the enhanced expression of antiapoptosis-related proteins and the strengthening depolymerization of microtubules in tumour cells [20]. However, a study on oral cancer revealed contrary results, i.e. that OLA1 regulates the EMT process negatively [10]. These data demonstrate that the relationship between OLA1 and EMT may be cancer type specific. The present study found that OLA1 expression was positively related with Snail, which is the key positive regulation molecule of EMT, indicating the potential role of OLA1 contributing to EMT in GC. Moreover, the combination of OLA1 and Snail reveals improved prognostic accuracy for GC patients, and it may be used as a select criterion for risk factor-stratified patient management. Nevertheless, the association of OLA1 and EMT in GC needs to be further explored in experiments.

Conclusions

We demonstrated that OLA1 expression was elevated in GC tissues both in mRNA and protein level, and was positively related to tumour size, and LNM and TNM stage. Moreover, OLA1 expression predicts shorter overall survival time and could be a candidate independent prognostic marker for GC patients. In addition, OLA1 expression was positively related with Snail, a key regulator of the EMT process, and the combination of them reveals improved prognostic accuracy for GC patients. These results suggested that OLA1 was recognized as a novel biomarker for prognosis and a potential therapeutic target for the treatment of GC.

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The authors declare no conflict of interest.

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