



# Mining the role of angiotensin-like protein family in gastric cancer and seeking potential therapeutic targets by integrative bioinformatics analysis

Cheng Tang  | Erbao Chen | Ke Peng | Haiwei Wang  | Xi Cheng | Yan Wang | Shan Yu | Yiyi Yu | Yuehong Cui | Tianshu Liu

Department of Medical Oncology,  
Zhongshan Hospital Affiliated to Fudan  
University, Shanghai, PR China

## Correspondence

Tianshu Liu and Yuehong Cui, Department  
of Medical Oncology, Zhongshan Hospital  
Affiliated to Fudan University, 180#  
Fenglin Road, Xuhui District, Shanghai  
200032, PR China.  
Email: liutianshu1969@126.com and cui.  
yuehong@zs-hospital.sh.cn

## Funding information

National Key Research and Development  
Plan of China, Grant/Award Number:  
2017YFC1308902; The Science and  
Technology Commission of Shanghai  
Municipality, Grant/Award Number:  
15411961900

## Abstract

**Background:** The indistinctive effects of antiangiogenesis agents in gastric cancer (GC) can be attributed to multifaceted gene dysregulation associated with angiogenesis. Angiotensin-like (ANGPTL) proteins are secreted proteins regulating angiogenesis. They are also involved in inflammation and metabolism. Emerging evidences have revealed their various roles in carcinogenesis and metastasis development. However, the mRNA expression profiles, prognostic values, and biological functions of ANGPTL proteins in GC are still elucidated.

**Methods:** We compared the transcriptional expression levels of ANGPTL proteins between GC and normal gastric tissues using ONCOMINE and TCGA-STAD. The prognostic values were evaluated by LinkedOmics and Kaplan–Meier Plotter, while the association of expression levels with clinicopathological features was generated through cBioPortal. We conducted the functional enrichment analysis with Metascape.

**Results:** The expression of ANGPTL1/3/6 was lower in GC tissues than in normal gastric tissues. High expression of ANGPTL1/2/4 was correlated with short overall survival and post-progression survival in GC patients. Upregulated ANGPTL1/2 was correlated with higher histological grade, non-intestinal Lauren classification, and advanced T stage, while ANGPTL4 exhibited high expression in early T stage, M1 stage, and non-intestinal Lauren classification.

**Conclusions:** Integrative bioinformatics analysis suggests that ANGPTL1/2/4 may be potential therapeutic targets in GC patients. Among them, ANGPTL2 acts as a GC promoter, while ANGPTL1/4's role in GC is still uncertain.

## KEYWORDS

angiotensin-like proteins, functional enrichment, gastric cancer, prognostic value, transcriptional expression level

Cheng Tang and Erbao Chen are contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Cancer Medicine* published by John Wiley & Sons Ltd.

## 1 | INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer in the world and the second leading cause among death from cancer. Taking the significant role of angiogenesis during cancer development into consideration, antiangiogenesis agents are expected to improve GC patients' prognosis significantly. However, so far, only vascular endothelial growth factor receptor (VEGFR) antibody ramucirumab<sup>1</sup> and VEGFR-targeted tyrosine kinase inhibitor (TKI) apatinib<sup>2</sup> have showed slight advantage of survival in the second- and third-line therapy of advanced GC, respectively. This phenomenon can be attributed to multifaceted gene dysregulation and complex molecular mechanisms associated with angiogenesis. Thus, figuring out other novel biomarkers related to angiogenesis process in GC may be helpful to improve the precision and efficacy of therapies.

Angiopoietin-like (ANGPTL) proteins are a family of secreted glycoproteins structurally similar to the angiopoietins.<sup>3</sup> To date, eight ANGPTL proteins have been discovered, namely from ANGPTL1 to ANGPTL8. Both angiopoietin and ANGPTL protein family are characterized by two domains: an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain.<sup>3</sup> Although the sequence is similar, ANGPTL proteins do not bind to the same receptors, named Tie2 or Tie1, as angiopoietins do. Instead, some of them bind to other kinds of receptors, such as leukocyte immune-globulin-like receptor B2 (LILRB2),<sup>4</sup> integrins  $\alpha 5\beta 1$ ,<sup>5</sup> and some of them are orphan ligands.<sup>4</sup>

Angiopoietins/Tie receptor signaling is involved in modulating angiogenesis and preserving vascular integrity and permeability.<sup>6</sup> Notwithstanding, none of the ANGPTL proteins bind to the angiopoietin receptors, most members still show effects on angiogenesis.<sup>7</sup> ANGPTL proteins also exhibit many other biological properties in lipid, glucose and energy metabolism, inflammation, hematopoiesis, as well as cancer progression and metastasis.<sup>3,7,8</sup>

ANGPTL proteins are widely expressed in many organs, such as skin, liver, breast, and gastrointestinal (GI) tract.<sup>3,8</sup> Researchers have discovered that ANGPTL proteins' transcriptional expression affected prognosis of patients in multiple types of cancer including lung cancer,<sup>9</sup> colorectal cancer (CRC),<sup>10</sup> liver cancer,<sup>11</sup> etc However, the influences can be quite different across different types of ANGPTL proteins and cancers. As far as we know, only ANGPTL2<sup>12,13</sup> and ANGPTL4<sup>14,15</sup> have been discussed previously in GC patients. Therefore, we consider that it is necessary to investigate ANGPTL proteins' transcriptional expression level across different clinicopathological situations and its relationship with prognosis of GC patients, thoroughly. In the present study, we implemented a deep bioinformatics analysis of ANGPTL proteins' mRNA expression data together with available clinical data of GC

patients based on several large public databases in order to illustrate their prognostic and potential therapeutic values in the treatment of GC.

## 2 | MATERIALS AND METHODS

### 2.1 | ONCOMINE data-mining analysis

ONCOMINE ([www.oncomine.org](http://www.oncomine.org)), an online web-based cancer database of RNA and DNA sequences, was used to facilitate data mining the transcriptional expression level of ANGPTL proteins in 20 types of cancer.<sup>16</sup> Transcriptional expression level of ANGPTL proteins in GC samples was compared with those in normal gastric samples using Student's *t*-test. Statistically significant *P* value and fold change (FC) were demarcated as  $P < .05$  and  $FC > 2$ , respectively.

### 2.2 | TCGA-STAD dataset

The Cancer Genome Atlas (TCGA) (<https://cancergenome.nih.gov>) contains gene expression data obtained by sequencing and has accurate clinicopathological data of many cancers. The stomach adenocarcinoma (STAD) dataset contains data from 375 GC tissues and 32 normal gastric tissues. The expression level of ANGPTL proteins was described using  $\text{Log}_2(\text{counts})$  together with 95% confidence interval (CI). Kruskal–Wallis (K–W) test was applied to compare expression level of different ANGPTL proteins. Further Dunn's multiple comparisons test would be conducted if the results of K–W test were of significance.  $P < .05$  was considered to be significant. Transcriptional expression of ANGPTL proteins in GC samples was compared with those in normal gastric samples using R statistical software package (<http://www.R-project.org>). *P* value  $< .01$  and Log FC absolute value greater than 1 were considered as filter to find differentially expressed ANGPTL proteins.

### 2.3 | LinkedOmics

The prognostic value of ANGPTL proteins' mRNA transcription level was measured using an online portal, the LinkedOmics ([www.linkedomics.org](http://www.linkedomics.org)),<sup>17</sup> which included gene expression profiles and clinical information of 375 GC patients from TCGA-STAD. Patients with GC were separated into two groups based on median gene expression (high vs low). The overall survival (OS) of these two groups were compared by Cox regression analysis and demonstrated with Kaplan–Meier (K–M) survival curves. The hazard ratio (HR) and *P* value were calculated as well.  $P < .05$  was considered significant.

## 2.4 | Kaplan–Meier plotter

The prognostic value of ANGPTL proteins' mRNA transcription level was also measured using an online open database, the Kaplan–Meier Plotter ([www.kmplot.com](http://www.kmplot.com)),<sup>18</sup> which included gene expression profiles and survival information of 876 GC patients from six other datasets rather than TCGA. Patients with GC were separated into two groups based on median gene expression (high vs low). The OS and post-progression survival (PPS) of these two groups were compared by a Kaplan–Meier survival plot. The HR with 95% CI and Log-rank *P* value were calculated. *P* < .05 was considered significant. We demonstrated K–M survival curves based on the value of each ANGPTL protein's most detected probe, and the number at risk was displayed below the curves.

## 2.5 | cBioPortal

The cBioPortal ([www.cbioportal.org](http://www.cbioportal.org)) is an open-access dataset for exploring multiple cancer genes. The GC dataset contains data from 415 cases, with pathologic diagnosis chosen by cBioPortal for further analyses of ANGPTL proteins.<sup>19,20</sup> The mRNA expression level of ANGPTL proteins in two groups of GC patients with different clinicopathological features was compared by Mann-Whitney (M-W) test. K-W test was applied in multiple groups comparison. Further Dunn's multiple comparisons test would be conducted if the results of K-W test were of significance. *P* < .05 was considered significant. Besides, genes with the highest expression correlation with each ANGPTL protein were generated by cBioPortal, and the top 120 co-expressed genes with highest Spearman correlation score were included in the following functional enrichment analysis.

## 2.6 | Metascape

Functional enrichment analysis was carried out using Metascape (<http://metascape.org>)<sup>21</sup> and analyzed in context of gene ontology (GO)<sup>22</sup> and Kyoto Encyclopedia of Genes and Genomes (KEGG)<sup>23</sup> biological pathways. The genes were assigned to functional groups based on molecular functions, biological processes, and specific pathways.

## 3 | RESULTS

### 3.1 | Downregulated expression of ANGPTL1/3/6 in patients with GC

Seven ANGPTL proteins were identified using the ONCOMINE database,<sup>16</sup> excluding ANGPTL8. As shown in Figure 1, we

first measured the expression of ANGPTL proteins in 20 types of cancer samples and compared those to normal tissue samples. The mRNA expression of ANGPTL1/2/3/6 was significantly dysregulated in GC samples in multiple datasets.

In accordance with Table 1, ANGPTL1's downregulation was observed in GC tissues compared with normal tissues, with a FC of  $-2.313$  in Cui's dataset<sup>24</sup> and a FC of  $-3.118$  in DErrico's dataset,<sup>25</sup> respectively. Overexpression of ANGPTL2 has been reported in diffuse gastric adenocarcinoma compared with normal gastric tissue, according to Chen<sup>26</sup> (FC = 2.239) and DErrico's (FC = 2.360) dataset, while ANGPTL2 was also upregulated in gastric cancer (FC = 2.249) according to Wang's dataset.<sup>27</sup> mRNA expression of ANGPTL3 was found to be downregulated in many types of GC compared to normal gastric tissues. Both Cho<sup>28</sup> (FC =  $-2.092$ ) and DErrico<sup>25</sup> (FC =  $-3.247$ ) exhibited elevating transcriptional level of ANGPTL3 in mixed-type gastric adenocarcinoma. Markedly decreased expression of ANGPTL3 was reported in diffuse-type GC and intestinal-type GC, with FC =  $-2.090$  and  $-3.599$ , by Cho and DErrico, respectively. In different datasets for ANGPTL3, we observed gastric adenocarcinoma having a FC =  $-2.074$  compared with normal stomach reported by Cho,<sup>28</sup> and a similar trend was found in Cui<sup>24</sup> (FC =  $-3.703$ ) and Wang's<sup>27</sup> (FC =  $-4.953$ ) datasets. Data from DErrico<sup>25</sup> reported downregulated ANGPTL6 level in intestinal-type gastric adenocarcinoma (FC =  $-3.797$ ).

Besides the datasets included by ONCOMINE, the transcriptional expression level of ANGPTL proteins in TCGA-STAD dataset is also demonstrated in Table 2, Figure 2A and B. ANGPTL1/2/4 exhibited much higher expression level than other members of ANGPTL family no matter in gastric cancer samples or in normal gastric samples (Data not shown, all *P* values generated by Dunn's multiple comparisons test were less than .05). The mean expression level [measured by  $\text{Log}_2(\text{counts})$ ] of ANGPTL1/2/4 was 9.982, 11.690, and 9.548 in normal tissue and 7.142, 11.930, and 8.792 in cancer tissue, respectively. When *P* value < .01 and Log FC absolute value greater than 1 were considered as filter, downregulated genes were ANGPTL1 ( $P = 4.480 \times 10^{-21}$ ), ANGPTL3 ( $P = 4.140 \times 10^{-5}$ ), ANGPTL4 ( $P = 3.882 \times 10^{-7}$ ), ANGPTL5 ( $P = 1.490 \times 10^{-9}$ ), ANGPTL6 ( $P = 4.150 \times 10^{-18}$ ), and ANGPTL7 ( $P = 1.600 \times 10^{-25}$ ). Therefore, both ONCOMINE database and TCGA-STAD dataset indicated that ANGPTL1/3/6 were downregulated in gastric cancer samples (Figure 3).

### 3.2 | Upregulated ANGPTL1/2/4 were correlated with poor prognosis in GC patients

We separated all GC patients into two groups (high vs low) based on median expression values for each ANGPTL

**FIGURE 1** Significantly changed ANGPTL protein's expression in different types of cancers. This information is attained from ONCOMINE and indicates the numbers of datasets with statistically significant ( $P$  value  $\leq 10^{-4}$ , Fold Change  $\geq 2$  and Gene Rank  $\geq$  Top 10%) mRNA high expression (Red) or low expression (Blue) of ANGPTL proteins (different types of cancer vs corresponding normal tissue). Cell color shade was decided by the best gene rank for the analyses within the cell, and the gene rank was analyzed by percentile of target genes in the top of all genes measured by each study

Analysis Type by Cancer	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal	Cancer vs. Normal
	ANGPTL1	ANGPTL2	ANGPTL3	ANGPTL4	ANGPTL5	ANGPTL6	ANGPTL7
Bladder Cancer		3					
Brain and CNS Cancer	1	4		2			2
Breast Cancer	6	1	13		12	5	
Cervical Cancer	1						
Colorectal Cancer	14	3		1		1	
Esophageal Cancer		1			2		2
Gastric Cancer	1	2		6			1
Head and Neck Cancer	2						
Kidney Cancer	2	4	1	1	3		3
Leukemia							
Liver Cancer	1			2	1		1
Lung Cancer	4						
Lymphoma		2					
Melanoma			1				
Myeloma				1	2		
Other Cancer	2	3					
Ovarian Cancer	1		1				
Pancreatic Cancer	1	2					
Prostate Cancer					3		3
Sarcoma		1	1	1			
Significant Unique Analyses	36	22	20	11	6	20	6
Total Unique Analyses	202	303	253	219	50	157	219

**TABLE 1** Differential expression of ANGPTL proteins between different types of GC and normal gastric tissue. (ONCOMINE)

	Type	Fold change	$P$ value	$t$ -test	REF
ANGPTL1	Gastric cancer	-2.313	$1.22 \times 10^{-5}$	-4.367	Cui
	Gastric cancer	-3.118	$5.37 \times 10^{-4}$	-3.568	DErrico
ANGPTL2	Gastric cancer	2.249	$3.36 \times 10^{-5}$	4.951	Wang
	Diffuse gastric adenocarcinoma	2.239	$5.94 \times 10^{-5}$	5.626	Chen
	Diffuse gastric adenocarcinoma	2.360	$1.00 \times 10^{-3}$	4.603	DErrico
ANGPTL3	Gastric adenocarcinoma	-2.074	$3.00 \times 10^{-6}$	-6.020	Cho
	Gastric mixed adenocarcinoma	-2.092	$1.12 \times 10^{-6}$	-6.607	Cho
	Diffuse gastric adenocarcinoma	-2.090	$1.19 \times 10^{-6}$	-6.659	Cho
	Gastric mixed adenocarcinoma	-3.247	$1.26 \times 10^{-5}$	-5.714	DErrico
	Gastric intestinal-type adenocarcinoma	-3.599	$6.11 \times 10^{-7}$	-5.459	DErrico
	Gastric cancer	-3.703	$4.81 \times 10^{-6}$	-4.574	Cui
	Gastric cancer	-4.953	$1.00 \times 10^{-3}$	-3.496	Wang
ANGPTL6	Gastric intestinal-type adenocarcinoma	-3.797	$1.68 \times 10^{-9}$	-7.084	DErrico

GC, gastric cancer  $t$ -test, Student's  $t$  test; REF, reference.

protein across all GC samples. As shown in Table 3 and Figure 4, seven ANGPTL proteins were identified using the LinkedOmics,<sup>17</sup> excluding ANGPTL8. Cox regression test of survival data indicated that increased ANGPTL1 ( $\geq 5.119$ ) [ $P = 5.731 \times 10^{-3}$ ], ANGPTL2 ( $\geq 10.456$ ) [ $P = 1.467 \times 10^{-2}$ ], ANGPTL4 ( $\geq 7.062$ ) [ $P = 1.799 \times 10^{-2}$ ], ANGPTL5 ( $\geq 0.540$ ) [ $P = 1.139 \times 10^{-2}$ ], and ANGPTL7

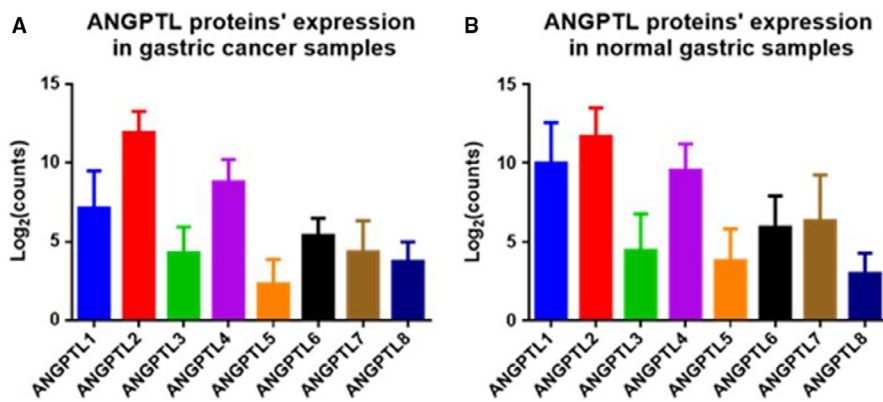
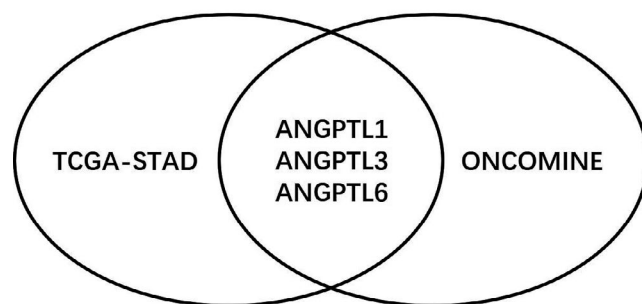
( $\geq 2.174$ ) [ $P = 3.051 \times 10^{-2}$ ] mRNA levels are associated with poor overall survival (OS) of GC.

We also investigated the prognostic value of ANGPTL proteins' expression level using Kaplan–Meier Plotter<sup>18</sup> and four members of the family could be identified. High expression of ANGPTL1 ( $\geq 27$ ) predicted poor OS in 631 patients (HR: 2.17 [1.74, 2.72];  $P: 3.2 \times 10^{-12}$ ). Similar situations

**TABLE 2** Differential expression of ANGPTL proteins between GC samples and normal gastric samples. (TCGA-STAD)

Name	Log <sub>2</sub> (counts) in normal sample	Log <sub>2</sub> (counts) in cancer sample	Log FC	<i>P</i> Value	FDR
<b>ANGPTL1</b>	9.982 [9.049, 10.910]	7.142 [6.902, 7.382]	-2.772	<b>4.480 × 10<sup>-21</sup></b>	2.210 × 10 <sup>-19</sup>
ANGPTL2	11.690 [11.030, 12.340]	11.930 [11.790, 12.070]	-0.472	2.266 × 10 <sup>-2</sup>	4.347 × 10 <sup>-2</sup>
<b>ANGPTL3</b>	4.434 [3.579, 5.290]	4.294 [4.127, 4.461]	-1.217	<b>4.140 × 10<sup>-5</sup></b>	1.780 × 10 <sup>-4</sup>
<b>ANGPTL4</b>	9.548 [8.950, 10.150]	8.792 [8.648, 8.937]	-1.180	<b>3.882 × 10<sup>-7</sup></b>	2.612 × 10 <sup>-6</sup>
<b>ANGPTL5</b>	3.792 [2.990, 4.595]	2.307 [2.129, 2.486]	-1.903	<b>1.490 × 10<sup>-9</sup></b>	1.552 × 10 <sup>-8</sup>
<b>ANGPTL6</b>	5.917 [5.187, 6.647]	5.386 [5.273, 5.498]	-1.729	<b>4.150 × 10<sup>-18</sup></b>	1.610 × 10 <sup>-17</sup>
<b>ANGPTL7</b>	6.324 [5.270, 7.379]	4.355 [4.154, 4.556]	-2.799	<b>1.600 × 10<sup>-25</sup></b>	1.31 × 10 <sup>-23</sup>
ANGPTL8	2.969 [2.484, 3.453]	3.744 [3.618, 3.870]	0.303	9.349 × 10 <sup>-2</sup>	1.442 × 10 <sup>-1</sup>

The bold text in first column highlights differentially expressed ANGPTL proteins ( $P < .01$ , absolute value of log FC > 1). The bold  $P$  values mean  $P < .01$ . FC, fold change; FDR, false discovery rate; GC, gastric cancer.

**FIGURE 2** ANGPTL proteins' expression level in TCGA-STAD dataset. (A) ANGPTL proteins' expression level in GC samples. (B) ANGPTL proteins' expression level in normal gastric samples. GC: gastric cancer**Differential expression of ANGPTLs between normal tissue and cancer tissue****FIGURE 3** Differentially expressed ANGPTL proteins indicated by both TCGA-STAD and ONCOMINE. GC: gastric cancer

were observed in patients with ANGPTL2 counts  $\geq 807$  and ANGPTL3 counts  $\geq 19$ , featuring a HR of 1.65 [1.39, 1.96] and a HR of 1.26 [1.01, 1.56], respectively. ANGPTL4 ( $\geq 100$ ) predicted short OS (HR: 1.17 [0.99, 1.38]) with a marginal significance ( $P = 7.1 \times 10^{-2}$ ) [Table 4(a) and Figure 5(A)]. In regard to ANGPTL1/2/3/4 expression's relationship with PPS, association similar to that with OS was found through K–M Plotter [Table 4(b) and Figure 5(B)]. Moreover, in 320

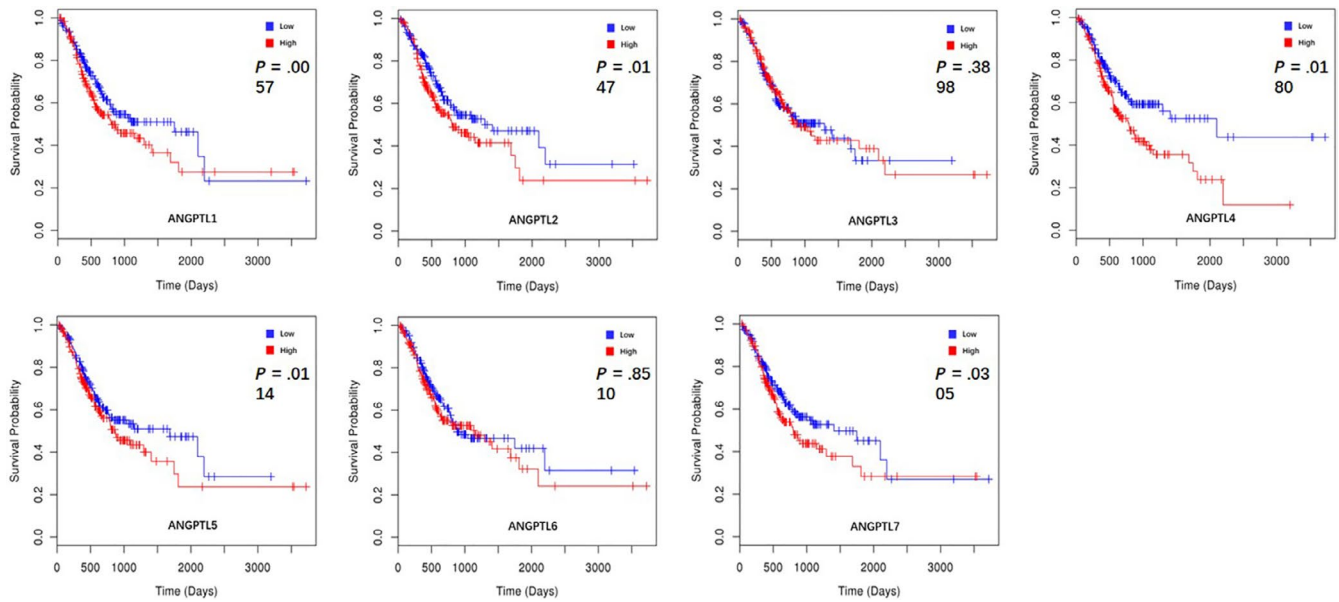
**TABLE 3** Cox regression revealed the association of ANGPTL proteins' expression with prognosis in GC patients (LinkedOmics)

Name	Cutoff value [Log <sub>2</sub> (RSEM)]	<i>P</i> value	FDR (BH)
<b>ANGPTL1</b>	5.119	<b>5.731 × 10<sup>-3</sup></b>	1.375 × 10 <sup>-2</sup>
<b>ANGPTL2</b>	10.456	<b>1.467 × 10<sup>-2</sup></b>	4.401 × 10 <sup>-2</sup>
ANGPTL3	1.342	3.898 × 10 <sup>-1</sup>	5.847 × 10 <sup>-1</sup>
<b>ANGPTL4</b>	7.062	<b>1.799 × 10<sup>-2</sup></b>	1.248 × 10 <sup>-1</sup>
<b>ANGPTL5</b>	0.540	<b>1.139 × 10<sup>-2</sup></b>	1.367 × 10 <sup>-1</sup>
ANGPTL6	3.421	8.510 × 10 <sup>-1</sup>	8.510 × 10 <sup>-1</sup>
<b>ANGPTL7</b>	2.174	<b>3.051 × 10<sup>-2</sup></b>	5.525 × 10 <sup>-2</sup>

The bold text in the first column highlights ANGPTL proteins with  $P < .05$ . The bold  $P$  values mean  $P < .05$ .

Cutoff value, median expression level; FDR (BH), FDR is calculated by BH (Benjamini-Hochberg method); FDR, false discovery rate; GC, gastric cancer;  $P$  value, generated by Cox regression test.

patients with intestinal-type GC, upregulated ANGPTL4 ( $\geq 95$ ) was related to poor OS with significance (HR: 1.53 [1.11, 2.10];  $P: 8.9 \times 10^{-3}$ ) [Figure 5(C)]. Therefore, we concluded that upregulated ANGPTL1/2/4 were correlated with poor prognosis in GC patients based on data from LinkedOmics and Kaplan–Meier Plotter [Figure 6].



**FIGURE 4** K–M curves revealed the association between OS and ANGPTL protein's expression in GC patients (LinkedOmics). *P*: generated by Cox's regression test; Low: patients with ANGPTL protein's expression lower than median expression level; High: patients with ANGPTL protein's expression higher than median expression level; K–M: Kaplan–Meier; OS: overall survival; GC: gastric cancer

**TABLE 4** Association of ANGPTL proteins' expression with prognosis in GC patients revealed by K–M Plotter

<b>(a) Upregulated ANGPTL1/2/4 were correlated with short OS in GC patients</b>					
Name	Cutoff value (counts)	Range (counts)	<i>P</i> value	HR	No. patients
ANGPTL1	27	1-3573	<b><math>3.2 \times 10^{-12}</math></b>	2.17 [1.74, 2.72]	631
ANGPTL2	807	47-6404	<b><math>7.7 \times 10^{-9}</math></b>	1.65 [1.39, 1.96]	876
ANGPTL3	19	0-295	<b><math>3.8 \times 10^{-2}</math></b>	1.26 [1.01, 1.56]	631
ANGPTL4	100	2-1564	<b><math>7.1 \times 10^{-2}</math></b>	1.17 [0.99, 1.38]	876
<b>(b) Up-regulated ANGPTL1/2/4 were correlated with short PPS in GC patients</b>					
ANGPTL1	42	0-3859	<b><math>1.3 \times 10^{-5}</math></b>	1.84 [1.39, 2.44]	384
ANGPTL2	644	90-6182	<b><math>3.1 \times 10^{-6}</math></b>	1.69 [1.35, 2.12]	499
ANGPTL3	22	1-3085	<b><math>2.3 \times 10^{-3}</math></b>	1.41 [1.13, 1.75]	499
ANGPTL4	95	5-1564	<b><math>6.2 \times 10^{-2}</math></b>	1.23 [0.99, 1.54]	499

The bold values in fourth column represent *P* values < .05.

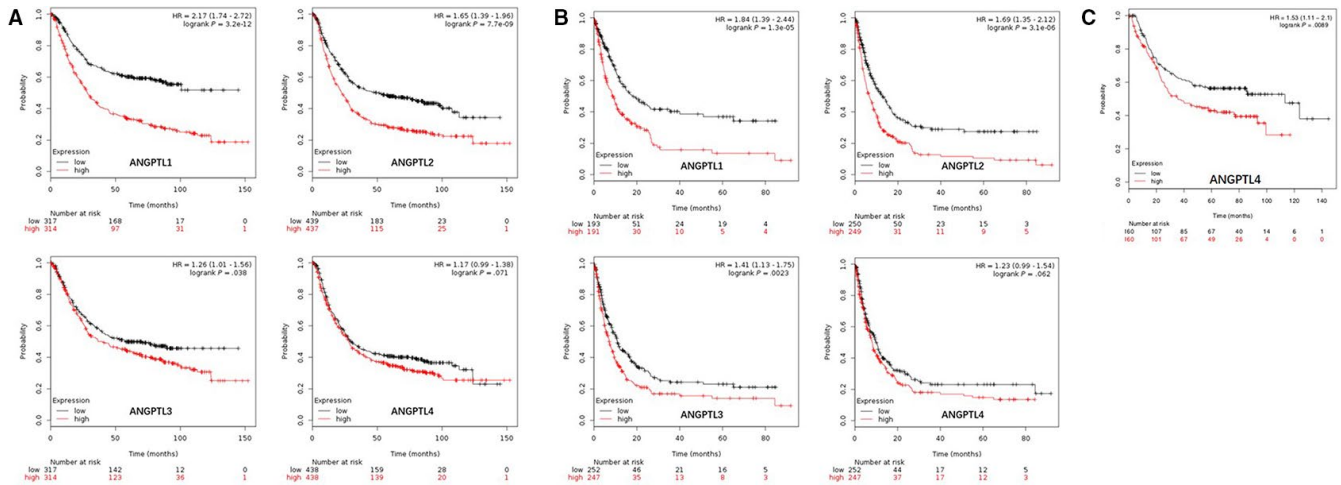
Cutoff value, median expression level; GC, gastric cancer; HR, hazard ratio; K–M Plotter, Kaplan–Meier plotter; No. Patients, number of patients; *P* value, Log rank *P* value.

### 3.3 | The association between ANGPTL1/2/3/4/6 expression level and clinicopathological features of GC patients

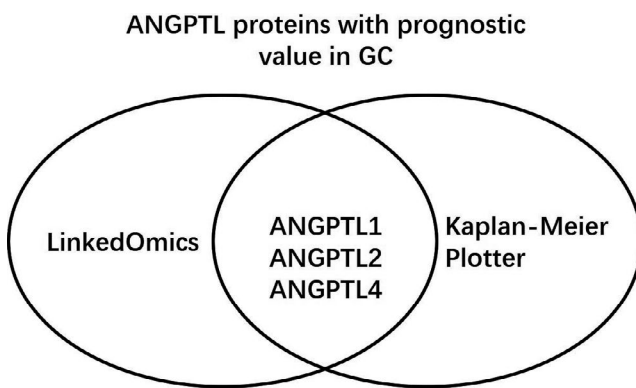
Taking ANGPTL proteins with aberrant expression or prognostic value into consideration, we next focused on the association between ANGPTL1/2/3/4/6 expression level and clinicopathological features of GC patients. M–W test was implemented to compare the mRNA expression level of ANGPTLs between two groups of GC patients from cBioPortal<sup>19,20</sup> with different clinicopathological features. Only ANGPTL4 exhibited lower expression in Asian than

in other races (*P* = .0016). For age criterion, there was no significant difference between <65 year and ≥65 year groups, except for ANGPTL1, which demonstrated reduced levels in the older group (*P* = .0231) (Figure 7A). No significant difference in ANGPTLs expression was observed across gender.

As shown in Figure 7(B), GC patients with higher histological grade (G3) showed higher expression of ANGPTL1 (*P* < .0001) and ANGPTL2 (*P* = .0002) than G1 and G2 patients. Intestinal-type GC patients tended to express low levels of ANGPTL1 (*P* < .0001), ANGPTL2 (*P* < .0001), and ANGPTL4 (*P* = .0357) compared to patients with mixed- and



**FIGURE 5** Upregulated ANGPTL1/2/4 were correlated with poor prognosis in GC patients according to K–M Plotter. (A) Kaplan-Meier curves revealed the OS differences based on mRNA level of ANGPTL proteins in GC patients. (B) Kaplan-Meier curves revealed the PPS differences based on mRNA level of ANGPTL proteins in GC patients. (C) Kaplan-Meier curves revealed the OS differences based on mRNA level of ANGPTL4 in intestinal-type GC patients. GC: gastric cancer; OS: overall survival; PPS: post-progression survival; K–M Plotter: Kaplan–Meier plotter; HR: hazard ratio; Low: patients with ANGPTL protein's expression lower than median expression level; High: patients with ANGPTL protein's expression higher than median expression level



**FIGURE 6** ANGPTL proteins with prognostic value in GC. High expression of ANGPTL1/2/4 predicted poor prognosis based on data from LinkedOmics and Kaplan-Meier Plotter. GC: gastric cancer

diffuse-type GC. The location of tumor lesion also affected the expression level of ANGPTL1 ( $P = .0030$ ), ANGPTL2 ( $P = .0014$ ), ANGPTL3 ( $P = .0100$ ), and ANGPTL4 ( $P = .0350$ ) according to K-W test. Further Dunn's multiple comparisons test revealed increased ANGPTL1 ( $P = .0033$ )/2 ( $P = .0012$ ) and decreased ANGPTL3 ( $P = .0071$ )/4 ( $P = .0030$ ) expression in antrum/distal group compared to gastroesophageal junction group (Data not shown).

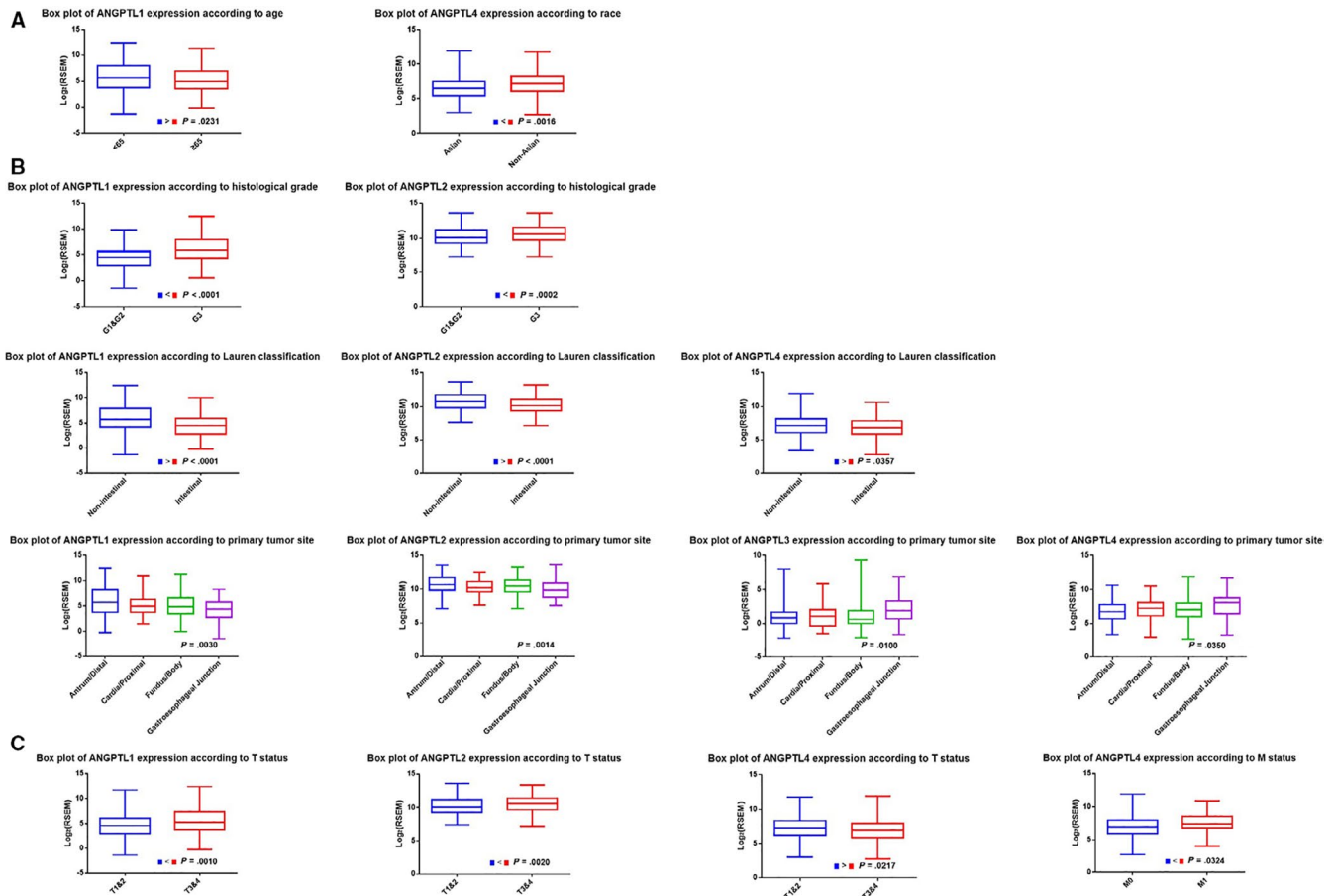
We found that the expression of ANGPTL1 ( $P = .0010$ ) and ANGPTL2 ( $P = .0020$ ) mRNA was significantly increased in T3 and T4 groups, while ANGPTL4 ( $P = .0217$ ) mRNA expression was significantly reduced in T3 and T4 groups. Upregulated ANGPTL4 was also found to be associated with metastasis ( $P = .0324$ ) (Figure 7C). Lymph node (N) status and clinical stage had no relationship with any of these ANGPTL protein's expression level.

### 3.4 | Functional enrichment analysis of genes co-expressed with ANGPTL1/2/4

Besides differential expression in cancer tissue and prognostic value, ANGPTL proteins' expression level in gastric tissue was also taken into account when we selected candidates for functional enrichment analysis. Since ANGPTL1/2/4 exhibited much higher expression level than other members of ANGPTL family in gastric tissue, we decided to further investigate their roles in GC genesis and development. The top 120 genes that had the most significant correlation with ANGPTL1/2/4 were generated by cBioPortal<sup>19,20</sup> and were included in the following functional enrichment analysis using Metascape.<sup>21</sup>

As shown in Figure 8(A), the top 120 genes co-expressed with ANGPTL1 were mainly enriched in molecular functions, biological processes, and pathways involved in interactions with extracellular matrix (ECM) (eg, glycosaminoglycan binding, keratan sulfate catabolic process, and ECM structural constituent and organization), cell differentiation and proliferation (eg, positive regulation of epithelial cell proliferation), tissue development (eg, mesenchyme development), and muscle system (eg, actin binding). Figure 8(B) was a network that exhibited the interactions among cluster of genes enriched in the molecular functions, biological processes, and pathways mentioned above. We could see that the genes enriched in ECM-related functions showed closer relationship with those enriched in cell differentiation and proliferation and tissue development-related processes.

According to Figure 8(C), ANGPTL2 probably participate in the development of tumor microenvironment, especially vasculature development. It may also regulate ECM



**FIGURE 7** ANGPTL proteins with significantly changed expression according to various clinicopathological features among ANGPTL1/2/3/4/6 (cBioPortal). (A) Differently expressed ANGPTL proteins according to demographical features; (B) Differently expressed ANGPTL proteins according to pathological features; (C) Differently expressed ANGPTL proteins according to clinical staging. Clinical staging was based on the 7<sup>th</sup> edition of AJCC TNM staging. Red boxes highlighted the box plots demonstrating significant difference between groups ( $P < .05$ ). GC: gastric cancer

binding and adhesion. Meanwhile, ANGPTL2 could possibly pose influences on cell proliferation and differentiation. Wnt signaling pathway and high mobility group box (HMG box) domain binding were among the top 20 functions enriched with ANGPTL2 co-expressed genes, too. Figure 8(D) illustrated an intimate relationship between the genes enriched in ECM organization- and adhesion-related functions and those enriched in vasculature development-related processes.

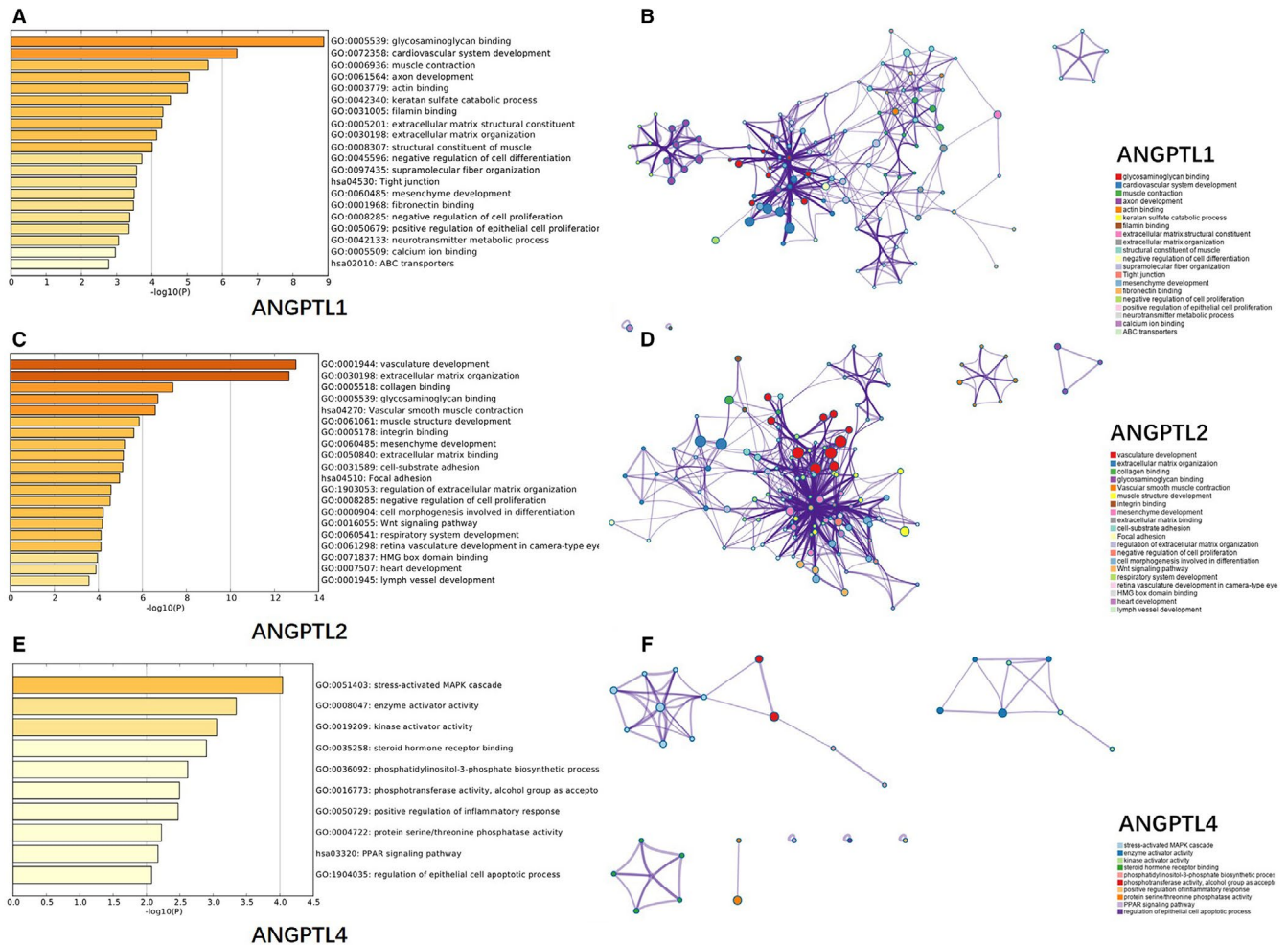
The genes co-expressed with ANGPTL4 did not enrich in some specific biological processes as remarkable as ANGPTL1/2 did. However, ANGPTL4 seemed to play a role in multiple different processes rather than only angiogenesis-related ones. It probably took part in the stress-activated mitogen-activated protein kinase cascade (MAPK cascade), peroxisome proliferator-activated receptor (PPAR) signaling pathway, phosphatidylinositol-3-phosphate (PI3P) biosynthetic process, epithelial cell apoptotic process, and regulation of inflammatory response. ANGPTL4 may also associate with activity as enzyme/kinase activator, phosphotransferase/phosphatase, or steroid hormone receptor binding process (Figure 8(E)). The network in Figure 8(F) revealed the

association between genes enriched in stress-activated MAPK cascade and those enriched in PI3P biosynthetic process, phosphotransferase activity function. Genes enriched in enzyme/kinase activator activity function only interacted with each other.

## 4 | DISCUSSION

ANGPTL proteins are a family of secreted glycoproteins which participate in multiple biological processes, mainly including angiogenesis, inflammation, and metabolism.<sup>3</sup> During the last decade, emerging evidences revealed ANGPTL proteins' roles in regulating different steps of carcinogenesis and metastasis through their effects on the processes mentioned above. Recently, Carbone et al<sup>3</sup> published a systematic review outlining the current knowledge about ANGPTL proteins' functions in angiogenesis, inflammation, cancer progression, and metastasis. They also discussed the most recent evidences sustaining ANGPTL proteins' role as prognostic biomarkers for cancer therapy. However, the roles of ANGPTL proteins in cancer progression and metastasis can be quite tumor type-dependent,





**FIGURE 8** Functional enrichment analysis of genes co-expressed with ANGPTL1/2/4 using Metascape. (A), (C), (E): Heatmaps of the molecular functions, biological processes, or pathways enriched with ANGPTL1/2/4 co-expressed genes. The bar color shade was decided by the  $P$  value, the deeper the shade, the less the  $P$  value. (B), (D), (F): Networks exhibiting interactions among the clusters of genes enriched in the molecular functions, biological processes, or pathways presented in the heatmaps (A), (C), (E), respectively. The points in different colors represented clusters of genes enriched in different molecular functions, biological processes, and pathways. The purple lines between points represented interactions between genes, the shorter the line, the closer the relationship. ABC transporters: ATP-binding cassette transporters; HMG box: high mobility group box; MAPK cascade: mitogen-activated protein kinase cascade; PPAR signaling pathway: peroxisome proliferator-activated receptor

even researches investigating the same type of cancer generate conflicting conclusions sometimes. So far, few articles have explored the mRNA expression of ANGPTL proteins in GC.<sup>12,14</sup> Their prognostic value and biological functions in GC remain to be elucidated, too. To our knowledge, this is the first study to exhibit ANGPTL proteins' transcriptional expression in GC comprehensively and to identify specific ANGPTL proteins with prognostic value, and biological functions in GC development using integrative bioinformatics.

#### 4.1 | ANGPTL proteins' mRNA expression in gastric tissues

Carbone et al have briefly summarized ANGPTL family members' mRNA expression level in esophageal and colorectal tissues based on previous published researches. ANGPTL1 was

highly expressed in all parts of the GI tract except esophagus.<sup>3</sup> ANGPTL2 exhibited high expression in esophageal<sup>3</sup> and colorectal cancer.<sup>10</sup> The expression level of ANGPTL4/6/7 was also high in CRC.<sup>29-31</sup> Our article revealed significantly higher expression level of ANGPTL1/2/4 than that of ANGPTL3/5/6/7 in gastric tissues through bioinformatics, which was not demonstrated in Carbone's review.<sup>3</sup> The information mentioned above together may provide us a rough impression of ANGPTL proteins' mRNA expression level landscape along the GI tract.

#### 4.2 | ANGPTL1: expression, prognostic value, and roles in GC

According to our results, both ONCOMINE database and TCGA-STAD dataset showed that ANGPTL1 were down-regulated in GC samples compared to normal gastric samples

and prompted ANGPTL1's potential inhibitory role in gastric tumorigenesis. Downregulation of ANGPTL1 was observed in kidney, lung, prostate, bladder, and thyroid cancers, too.<sup>3</sup> In fact, ANGPTL1 was generally supported to be a tumor suppressor across different types of tumor by both in vitro and in vivo experiments. For example, ANGPTL1 overexpression in breast cancer (BC) cells could result in a significant reduction in the number and size of tumor nodules.<sup>32</sup> Primary melanoma tumors derived from ANGPTL1-secreting cells grew more slowly in vivo compared to empty vector-transfected cells.<sup>32</sup> It has also been reported that ANGPTL1 treatment remarkably inhibited in vitro and in vivo migration and invasion ability of hepatocellular carcinoma (HCC) cells.<sup>11</sup> Meanwhile, immunohistochemistry (IHC) analysis of HCC samples revealed that patients with higher level of ANGPTL1 expression had less metastasis as well as longer survival time.<sup>11</sup>

Several possible mechanisms underlie this suppressive activity. Firstly, ANGPTL1 may play an essential role in tumor inhibition by balancing angiogenesis and permeability. It could both inhibit VEGF-induced endothelial cells proliferation and induce extracellular signal-regulated kinase 1/2 (ERK1/2)-related antiapoptotic activity.<sup>32</sup> Secondly, ANGPTL1 was responsible for reorganization of cytoskeleton through inhibition of actin stress fiber formation, which probably result in an altered cellular morphology. Thirdly, ANGPTL1 could induce mesenchymal-to-epithelial transition (MET) through integrin  $\alpha1\beta1$ , miR-630, and SLUG (SNAIL-related zinc-finger transcription factor) pathway, thus allow cancer cells to regain epithelial properties.<sup>9</sup>

However, according to our results, upregulated ANGPTL1 was correlated with poor prognosis, higher histological grade, non-intestinal Lauren classification, and advanced T stage in GC patients suggesting a GC-promoting role of this molecule. Perhaps, ANGPTL1 played different roles in gastric tumorigenesis and gastric tumor progression. There was a lack of researches focusing on ANGPTL1's roles in GC. Our functional enrichment analysis indicated several pathways by which ANGPTL1 exerted influence on GC progression.

Most of the pathways are related to interactions with ECM (eg, glycosaminoglycan binding, keratan sulfate catabolic process, and ECM structural constituent and organization), cell differentiation and proliferation (eg, positive regulation of epithelial cell proliferation), tissue development (eg, mesenchyme development), and muscle system (eg, actin binding). Further studies could be conducted based on this analysis.

### 4.3 | ANGPTL2: expression, prognostic value, and roles in GC

ANGPTL2 has been proved to be tumor-promoting among several types of cancer. High ANGPTL2 expression level

has been observed in many types of cancer including esophageal,<sup>3</sup> colorectal,<sup>10</sup> prostate,<sup>5</sup> pancreatic,<sup>33</sup> lung,<sup>34</sup> breast,<sup>35</sup> liver,<sup>36</sup> and skin<sup>37</sup> cancers. ANGPTL2 mainly exerted its pro-angiogenic and antiapoptotic abilities in the tumor microenvironment. ANGPTL2 also increased cancer cells' migratory and invasive ability, thus facilitate tumor metastases through different mechanisms. For instance, an autocrine signaling between ANGPTL2 and its receptor LILRB2 was able to induce early EMT and tumor progression in preneoplastic pancreatic ductal cells.<sup>33</sup> ANGPTL2 also strengthened responsiveness of BC cells to chemokine (C-X-C motif) ligand 12 (CXCL12) by the upregulation of C-X-C motif receptor 4 (CXCR4), thus promoted these cells' recruitment to bone metastatic sites.<sup>35</sup> In addition, ANGPTL2 induced inflammation and oxidative stress, generating a tumor microenvironment that supported methylation, and consequently reducing gene expression of DNA repair enzymes, such as mutS homolog 2 (MSH2), leading to DNA mutations and cancer initiation in an experimental model of skin cancer.<sup>37</sup>

Our bioinformatics analysis revealed elevated expression of ANGPTL2 in GC tissue compared to normal gastric tissue based on data from ONCOMINE and no significant difference in ANGPTL2 expression between normal and cancer tissue based on data from TCGA-STAD. Besides, upregulated ANGPTL2 was also correlated with poor prognosis, higher histological grade, non-intestinal Lauren classification, and advanced T stage in GC patients according to our results. Therefore, we may conclude that ANGPTL2 behave as a tumor promoter in GC just like in many other types of cancer. Several studies on ANGPTL2 and GC generated similar conclusion as well. As in vitro researches indicated, ANGPTL2 knockdown caused anoikis and inhibited proliferation, invasion, and migration in GC cells,<sup>12</sup> while proliferation rate and invasive ability in ANGPTL2-overexpressed GC cells were higher than in control cells.<sup>13</sup> Besides, higher expression of ANGPTL2 was observed in highly malignant and undifferentiated GC cell lines.<sup>13,38</sup> Clinical researches prompted that upregulated ANGPTL2 was associated with GC progression, early recurrence, and poor prognosis.<sup>12</sup> Moreover, ANGPTL2 could be a potential novel noninvasive biomarker for GC. The serum ANGPTL2 level of GC patients were significantly higher than those of healthy controls. Recent studies reported receiver operating characteristic (ROC) curves which yielded robust AUC value (0.831) accompanied by high sensitivity (73.0%) and specificity (82.2%) in distinguishing GC patients from healthy controls.<sup>12</sup>

Although ANGPTL2's GC promotive activity was observed by both in vitro studies and clinical researches, few studies explored the possible underlying mechanisms. According to our functional analysis of co-expressed genes in GC, ANGPTL2 probably participate in the development of tumor microenvironment, especially vasculature development. It may also regulate ECM binding and adhesion.

Meanwhile, ANGPTL2 could possibly pose influences on cell proliferation and differentiation. Wnt signaling pathway and HMG box domain binding were among the top 20 functions enriched with ANGPTL2 co-expressed genes, too. These candidate pathways mentioned above needed further validation of experiments and could be hints of future mechanism studies.

#### 4.4 | ANGPTL3: expression and prognostic value in GC

Few articles about ANGPTL3's role in cancer growth and invasion were reported. Existing studies showed contradicting results in different types of cancer. For instance, ANGPTL3 was significantly upregulated in oral squamous cell carcinoma (OSCC)-derived cell lines compared to normal tissues. *In vitro* and *in vivo* OSCC models showed that ANGPTL3 knockdown arrested cell cycle at G1 phase through upregulating cyclin-dependent kinase inhibitors, thus reduced cancer cell proliferation and growth.<sup>39</sup> Nonetheless, in HCC cells, ANGPTL3 inhibited cell proliferation and invasion through downregulation of p38MAPK and MMP-9 cascade's activation.<sup>40</sup>

ANGPTL3 exhibited low expression in gastric tissues, though ANGPTL3 was downregulated in GC tissues compared to normal gastric tissues suggesting ANGPTL3's role as a GC suppressor. However, the difference in expression level did not make a difference in GC patients' prognosis and was not associated with other clinicopathological factors, except primary tumor site. Therefore, we are prone to the viewpoint that ANGPTL3 may not play an important role in regulating GC genesis and progression.

#### 4.5 | ANGPTL4: expression, prognostic value, and roles in GC

Recent researches revealed ANGPTL4's wide-spectrum of action including cancer growth, angiogenesis, metabolism, and metastasis. However, it seemed that ANGPTL4 acted in a tumor type-dependent manner and even the findings in the same type of cancer contradicted with each other sometimes, too.

For instance, ANGPTL4 could be induced by hypoxia through upregulation of PGE2 receptor in CRC, thus promoted cancer cell proliferation. It could also stimulate a redox-based mechanism which enhanced tumor cell survival by alteration of the O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub> ratio and led to the activation of extracellular signal-regulated kinase in CRC.<sup>14</sup> Inconsistent with upregulated expression in CRC, ANGPTL4 expression was significantly lower in HCC tissues than in nontumor tissues. Low expression of ANGPTL4 was significantly associated with advanced tumor stage, poor differentiation as

well as poor overall and disease-free survival (DFS) of HCC patients.<sup>15</sup> However, a study showed that serum ANGPTL4 protein is higher in HCC patients than in normal controls.<sup>41</sup> Besides, ANGPTL4 was reported to be expressed at higher level in the blood of BC patients<sup>42</sup> and high expression of ANGPTL4 correlated with a minor DFS of young BC patients.<sup>43</sup> However, an *in vitro* study indicated that PPAR  $\beta/\delta$ -regulated ANGPTL4 strongly inhibited the transforming growth factor  $\beta$  (TGF  $\beta$ )-induced invasion of MDA-MB-231 human BC cells.<sup>44</sup>

ANGPTL4's proteolytic cleavage generated two isoforms of itself: an N-terminal coiled-coil domain (nANGPTL4), and a large fibrinogen-like COOH-terminal domain (cANGPTL4). Whereas the former was mainly involved in the endocrine regulatory role of lipid metabolism, insulin sensitivity, and glucose homeostasis, the latter may be a key regulator of the complex signaling during cancer development.<sup>3</sup> The complexity of ANGPTL4's role in cancer genesis and development probably result from the alteration of cleavage and posttranslational modification.<sup>45</sup>

Our integrated bioinformatics analysis further demonstrated the complexity of ANGPTL4's role in gastric cancer. According to data from TCGA-STAD, ANGPTL4 exhibited lower expression in GC tissue than in normal gastric tissue and higher expression in early T stage than advanced T stage. However, elevated ANGPTL4 expression level was also correlated with poor prognosis, unfavorable Lauren classification, and metastasis in GC patients. Previous researches focusing on ANGPTL4 and GC could not provide consistent conclusion, neither. Kubo et al suggested that hypoxia-induced ANGPTL4 expression is independent of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) in hypoxic GC cells and ANGPTL4 may be a favorable marker for predicting a long survival time.<sup>46</sup> Meanwhile, Baba et al demonstrated that hypoxia-induced ANGPTL4 expression was regulated by HIF-1 $\alpha$  in scirrhous GC cells and was essential for tumor growth, metastasis, and resistance to anoikis through different mechanisms, including downregulation of c-Myc and focal adhesion kinase (FAK)/Src/phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt)/ERK pathway, upregulation of p27, and apoptotic factors caspases-3, -8, and -9.<sup>47</sup> Besides, Tan et al found that cANGPTL4 bearing T266M mutation (T266M cANGPTL4) bound to integrin  $\alpha 5\beta 1$  with a reduced affinity compared to wild-type cANGPTL4, leading to weaker activation of downstream signaling molecules. The tumors with T266M cANGPTL4 exhibited impaired proliferation, anoikis resistance, migratory capability, and had reduced adenylate energy charge. Further investigations also revealed that cANGPTL4 regulated the expression of glucose transporter 2 (Glut2).<sup>48</sup>

Many of the mentioned pathways by which ANGPTL4 exerted influence on other types of cancer were enriched in our functional enrichment analysis of ANGPTL4 co-expressed

genes in GC, such as stress-activated MAPK cascade, PPAR signaling pathway, PI3P biosynthetic process, epithelial cell apoptotic process, and regulation of inflammatory response. However, some of them have not been verified by experiments in GC, perhaps further researches are needed.

#### 4.6 | ANGPTL6: expression and prognostic value in GC

Similar to ANGPTL3, ANGPTL6 also showed low expression in gastric tissues and was downregulated in GC tissues. Based on our results, ANGPTL6's expression level did not exhibit prognostic value in GC patients and was not associated with other clinicopathological factors, neither. All the information seemed to suggest that ANGPTL6 may not exert much influence on GC genesis and progression.

However, it has been reported that the interaction between hepatic ANGPTL6 and tumoral integrin/E-cadherin drives liver homing and colonization by CRC cells. Furthermore, an angiopoietin-like 6-mimicking peptide was capable of interfering with this interaction, thus acting as an antimetastatic compound.<sup>30</sup> Hence, we guessed that it was still a possible research direction to investigate ANGPTL6's role in liver metastasis of GC.

#### 4.7 | ANGPTL1/2/4 and resistance of antiangiogenesis agents

Emerging evidences has already demonstrated ANGPTL proteins' potential roles in resistance of antiangiogenesis agents in other types of cancer. For example, a study indicated that ANGPTL1 could inhibit sorafenib resistance and cancer stemness in HCC cells through acting as a Met receptor inhibitor.<sup>49</sup> Besides, ANGPTL2 was proved to be among the pro-inflammatory factors overexpressed in pancreatic cancer (PC) cells which led to epithelial-to-mesenchymal transition (EMT) and resistance of anti-VEGF treatment.<sup>50,51</sup> Moreover, in a research on triple-negative breast cancer (TNBC), heparin-binding epidermal growth factor (HB-EGF) was found to play a pivotal role in the acquisition of tumor aggressiveness by regulating both ANGPTL4 and VEGFA.<sup>52</sup> We thought this research indicated that only blocking VEGFR may not be enough to shut down angiogenesis in TNBC. Whether the ANGPTL proteins participated in the mechanism of antiangiogenesis drug resistance in GC or not has not been studied thoroughly before.

## 5 | CONCLUSIONS

In the current study, we systematically analyzed transcriptional expression level and prognostic value of ANGPTL

proteins in GC patients. We also exhibited the association of expression level with clinicopathological features and supplied a functional enrichment analysis. Integrative bioinformatics analysis suggests that ANGPTL1/2/4, compared to other ANGPTL proteins, may be potential therapeutic targets in GC patients. Among ANGPTL1/2/4, ANGPTL2 tends to be a GC promoter according to our results. However, we cannot conclude whether ANGPTL1/4 are GC promoter or suppressor based on the diverse information provided by our analysis. The ANGPTL proteins' roles in GC are so complex that more well-conducted clinical researches and in-depth experiments are required to validate the diagnostic value of these ANGPTL proteins and explore the underlying mechanism by which ANGPTL proteins influence GC's development.

#### ACKNOWLEDGMENTS

This research was supported by (1) Study on the prevention and control of major chronic non-infectious diseases, National Key Research and Development Plan of China, 2017YFC1308902, and (2) The Science and Technology Commission of Shanghai Municipality, 15411961900.

#### CONFLICT OF INTEREST

The authors declare that they have no competing interests.

#### AUTHOR CONTRIBUTIONS

CT and EC conceived the project; CT, EC, HW, and KP analyzed the data, CT, XC, and YW drafted the manuscript; TL and YC had critically read the manuscript; SY and YY edited and reviewed the manuscript. All authors read and approved the final manuscript.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

#### DATA AVAILABILITY STATEMENT

The data utilized in this study are publicly available and listed below: ONCOMINE ([www.oncomine.org](http://www.oncomine.org)); TCGA (<https://cancergenome.nih.gov>); LinkedOmics ([www.linkedomics.org](http://www.linkedomics.org)); Kaplan–Meier Plotter ([www.kmplot.com](http://www.kmplot.com)); cBioPortal ([www.cbioportal.org](http://www.cbioportal.org)); Metascape (<http://metascape.org>).

#### ORCID

Cheng Tang  <https://orcid.org/0000-0002-5876-0315>

Haiwei Wang  <https://orcid.org/0000-0002-9675-4039>

#### REFERENCES

1. Wilke H, Muro K, Van Cutsem E, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-esophageal junction adenocarcinoma (RAINBOW): a double-blind, randomized phase 3 trial. *Lancet Oncol*. 2014;15:1224-1235.

2. Li J, Qin S, Xu J, et al. Randomized, double-Blind, placebo-Controlled phase III trial of apatinib in patients with chemotherapy-refractory advanced or metastatic adenocarcinoma of the stomach or gastroesophageal junction. *J Clin Oncol*. 2016;34:1448-1454.
3. Carbone C, Piro G, Merz V, et al. Angiopoietin-like proteins in angiogenesis, inflammation and cancer. *Int J Mol Sci*. 2018;19:E431. <https://doi.org/10.3390/ijms19020431>.
4. Deng MI, Lu Z, Zheng J, et al. A motif in LILRB2 critical for ANGPTL2 binding and activation. *Blood*. 2014;124:924-935.
5. Sato R, Yamasaki M, Hirai K, et al. Angiopoietin-like protein 2 induces androgen-independent and malignant behavior in human prostate cancer cells. *Oncol Rep*. 2015;33:58-66.
6. Jones N, Iljin K, Dumont DJ, Alitalo K. Tie receptors: new modulators of angiogenic and lymphangiogenic responses. *Nat Rev Mol Cell Biol*. 2001;2:257-267.
7. Hato T, Tabata M, Oike Y. The role of angiopoietin-like proteins in angiogenesis and metabolism. *Trends Cardiovasc Med*. 2008;18:6-14.
8. Santulli G. Angiopoietin-like proteins: a comprehensive look. *Front Endocrinol (Lausanne)*. 2014;23:4. <https://doi.org/10.3389/fendo.2014.00004>.
9. Kuo T-C, Tan C-T, Chang Y-W, et al. Angiopoietin-like protein 1 suppresses SLUG to inhibit cancer cell motility. *J Clin Invest*. 2013;123:1082-1095.
10. Toiyama Y, Inoue Y, Shimura T, et al. Serum Angiopoietin-like protein 2 improves preoperative detection of lymph node metastasis in colorectal cancer. *Anticancer Res*. 2015;35:2849-2856.
11. Yan Q, Jiang L, Liu M, et al. ANGPTL1 interacts with integrin  $\alpha 1\beta 1$  to suppress HCC angiogenesis and metastasis by inhibiting JAK2/STAT3 signaling. *Cancer Res*. 2017;77:5831-5845.
12. Toiyama Y, Tanaka K, Kitajima T, et al. Serum angiopoietin-like protein 2 as a potential biomarker for diagnosis, early recurrence and prognosis in gastric cancer patients. *Carcinogenesis*. 2015;36:1474-1483. <https://doi.org/10.1093/carcin/bgv139>.
13. Sheng WZ, Chen YS, Tu CT, He J, Zhang B, Gao WD. ANGPTL2 expression in gastric cancer tissues and cells and its biological behavior. *World J Gastroenterol*. 2016;22:10364-10370. <https://doi.org/10.3748/wjg.v22.i47.10364>.
14. Zhu P, Tan M, Huang R-L, et al. Angiopoietin-like 4 protein elevates the pro-survival intracellular O<sub>2</sub>-:H<sub>2</sub>O<sub>2</sub> ratio and confers anoikis resistance to tumors. *Cancer Cell*. 2011;19:401-415. <https://doi.org/10.1016/j.ccr.2011.01.018>.
15. Ng K-P, Xu A, Cheng Q, et al. Clinical relevance and therapeutic potential of angiopoietin-like protein 4 in hepatocellular carcinoma. *Mol Cancer*. 2014;13:196.
16. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia*. 2004;6:1-6.
17. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Res*. 2018;46:D956-D963. <https://doi.org/10.1093/nar/gkx1090>.
18. Lanczky A, Nagy A, Bottai G, et al. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2,178 breast cancer patients. *Breast Cancer Res Treat*. 2016;160:439-446.
19. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(Suppl):1.
20. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401-404.
21. Tripathi S, Pohl M, Zhou Y, et al. Meta- and orthogonal integration of influenza "OMICS" data defines a role for UBR4 in virus budding. *Cell Host Microbe*. 2015;18:723-735.
22. Gene ontology consortium. Gene ontology consortium: going forward. *Nucleic Acids Res*. 2015;43:D1049-D1056. <https://doi.org/10.1093/nar/gku1179>.
23. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res*. 2017;45:D353-D361. <https://doi.org/10.1093/nar/gkw1092>.
24. Cui J, Chen Y, Chou W-C, et al. An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer. *Nucleic Acids Res*. 2011;39:1197-1207.
25. D'Errico M, Rinaldis ED, Blasi MF, et al. Genome-wide expression profile of sporadic gastric cancers with microsatellite instability. *Eur J Cancer*. 2009;45:461-469.
26. Chen X, Leung SY, Yuen ST, et al. Variation in gene expression patterns in human gastric cancers. *Mol Biol Cell*. 2003;14:3208-3215.
27. Wang Q, Wen Y-G, Li D-P, et al. Upregulated INHBA expression is associated with poor survival in gastric cancer. *Med Oncol*. 2012;29:77-83.
28. Cho JY, Lim JY, Cheong JH, et al. Gene expression signature-based prognostic risk score in gastric cancer. *Clin Cancer Res*. 2011;17:1850-1857.
29. Huang XF, Han J, Hu XT, He C. Mechanisms involved in biological behavior changes associated with Angptl4 expression in colon cancer cell lines. *Oncol Rep*. 2012;27:1541-1547.
30. Marchiò S, Soster M, Cardaci S, et al. A complex of  $\alpha 6$  integrin and E-cadherin drives liver metastasis of colorectal cancer cells through hepatic angiopoietin-like 6. *EMBO Mol Med*. 2012;4:1156-1175.
31. Parri M, Pietrovito L, Grandi A, et al. Angiopoietin-like 7, a novel pro-angiogenic factor over-expressed in cancer. *Angiogenesis*. 2014;17:881-896. <https://doi.org/10.1007/s10456-014-9435-4>.
32. Dhanabal M, Jeffers M, LaRochelle WJ, Lichenstein HS. Angioarrestin: a unique angiopoietin-related protein with anti-angiogenic properties. *Biochem Biophys Res Commun*. 2005;333:308-315. <https://doi.org/10.1016/j.bbrc.2005.05.134>.
33. Carbone C, Piro G, Fassan M, et al. An angiopoietin-like protein 2 autocrine signaling promotes EMT during pancreatic ductal carcinogenesis. *Oncotarget*. 2015;6:13822-13834.
34. Sasaki H, Suzuki A, Shitara M, et al. Angiopoietin-like protein ANGPTL2 gene expression is correlated with lymph node metastasis in lung cancer. *Oncol Lett*. 2012;4:1325-1328.
35. Masuda T, Endo M, Yamamoto Y, et al. ANGPTL2 increases bone metastasis of breast cancer cells through enhancing CXCR4 signaling. *Sci Rep*. 2015;5:9170.
36. Gao L, Ge C, Fang T, et al. ANGPTL2 promotes tumor metastasis in hepatocellular carcinoma. *J Gastroenterol Hepatol*. 2015;30:396-404.
37. Endo M, Nakano M, Kadomatsu T, et al. Tumor cell-derived angiopoietin-like protein ANGPTL2 is a critical driver of metastasis. *Cancer Res*. 2012;72:1784-1794.
38. Yoshinaga T, Shigemitsu T, Nishimata H, Takei T, Yoshida M. Angiopoietin-like protein 2 is a potential biomarker for gastric cancer. *Mol Med Rep*. 2015;11:2653-2658.

39. Koyama T, Ogawara K, Kasamatsu A, et al. ANGPTL3 is a novel biomarker as it activates ERK/MAPK pathway in oral cancer. *Cancer Med.* 2015;4:759-769.
40. Yu H, Zhang H, Li D, et al. Effects of ANGPTL3 antisense oligodeoxynucleotides transfection on the cell growths and invasion of human hepatocellular carcinoma cells. *Hepatogastroenterology.* 2011;58:1742-1746.
41. Li H, Ge C, Zhao F, et al. Hypoxia-inducible factor 1 alpha-activated angiopoietin-like protein 4 contributes to tumor metastasis via vascular cell adhesion molecule-1/integrin  $\beta$ 1 signaling in human hepatocellular carcinoma. *Hepatology.* 2011;54:910-919.
42. Evans RL, Pottala JV, Eglund KA. Classifying patients for breast cancer by detection of autoantibodies against a panel of conformation-carrying antigens. *Cancer Prev Res.* 2014;7:545-555.
43. Johnson RH, Hu P, Fan C, Anders CK. Gene expression in "young adult type" breast cancer: a retrospective analysis. *Oncotarget.* 2015;6:13688-13702.
44. Adhikary T, Brandt DT, Kaddatz K, et al. Inverse PPAR $\beta$ / $\delta$  agonists suppress oncogenic signaling to the ANGPTL4 gene and inhibit cancer cell invasion. *Oncogene.* 2013;32:5241-5252.
45. Tan MJ, Teo Z, Sng MK, Zhu P, Tan NS. Emerging roles of angiopoietin-like 4 in human cancer. *Mol Cancer Res.* 2012;10:677-688. <https://doi.org/10.1158/1541-7786.MCR-11-0519>.
46. Kubo H, Kitajima Y, Kai K, et al. Regulation and clinical significance of the hypoxia-induced expression of ANGPTL4 in gastric cancer. *Oncol Lett.* 2016;11:1026-1034.
47. Baba K, Kitajima Y, Miyake S, et al. Hypoxia-induced ANGPTL4 sustains tumour growth and anoikis resistance through different mechanisms in scirrhous gastric cancer cell lines. *Sci Rep.* 2017;7:11127. <https://doi.org/10.1038/s41598-017-11769-x>.
48. Tan ZW, Teo Z, Tan C, et al. ANGPTL4 T266M variant is associated with reduced cancer invasiveness. *Biochim Biophys Acta.* 2017;1864:1525-1536. <https://doi.org/10.1016/j.bbamcr.2017.06.010>.
49. Chen H-A, Kuo T-C, Tseng C-F, et al. Angiopoietin-like protein 1 antagonizes met receptor activity to repress sorafenib resistance and cancer stemness in hepatocellular carcinoma. *Hepatology.* 2016;64:1637-1651. <https://doi.org/10.1002/hep.28773>.
50. Carbone C, Moccia T, Zhu C, et al. Anti-VEGF treatment-resistant pancreatic cancers secrete proinflammatory factors that contribute to malignant progression by inducing an EMT cell phenotype. *Clin Cancer Res.* 2011;17:5822-5832.
51. Gaianigo N, Melisi D, Carbone C. EMT and treatment resistance in pancreatic cancer. *Cancers (Basel).* 2017;9:E122. <https://doi.org/10.3390/cancers9090122>.
52. Yotsumoto F, Tokunaga E, Oki E, et al. Molecular hierarchy of heparin-binding EGF-like growth factor-regulated angiogenesis in triple-negative breast cancer. *Mol Cancer Res.* 2013;11:506-517.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Tang C, Chen E, Peng K, et al. Mining the role of angiopoietin-like protein family in gastric cancer and seeking potential therapeutic targets by integrative bioinformatics analysis. *Cancer Med.* 2020;9:4850–4863. <https://doi.org/10.1002/cam4.3100>