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In Silico Identification of Contradictory Role of ADAMTS5 in Hepatocellular Carcinoma

Zhipeng Zhu[1], Jiuhua Xu[2], Xiaofang Wu[2], Sihao Lin[1], Lulu Li[1], Weipeng Ye[2], and Zhengjie Huang[1,2]

Abstract

Background: *ADAMTS5* has different roles in multiple types of cancers and participates in various molecular mechanisms. However, the prognostic value of *ADAMTS5* in patients with hepatocellular carcinoma (HCC) still remains unclear. We carried the study to evaluate the prognostic value and identified underlying molecular mechanisms in HCC. **Methods:** Firstly, the association of *ADAMTS5* expression and clinicopathological parameters was evaluated by in GSE14520. Next, *ADAMTS5* expression in HCC was performed using GSE14520, GSE36376, GSE76427 and The Cancer Genome Atlas (TCGA) profile. Furthermore, Kaplan-Meier analysis, Univariate and Multivariate Cox regression analysis, subgroup analysis was performed to evaluate the prognostic value *of ADAMTS5* in HCC. Finally, GO enrichment analysis, gene set enrichment analysis (GSEA) and weighted gene co-expression network analysis (WGCNA) were performed to revealed underlying molecular mechanisms. **Result:** The expression of *ADAMTS5* was positively correlated with the development of HCC. Next, high *ADAMTS5* expression was significantly associated with poorer survival (all $P < 0.05$) and the impact of *ADAMTS5* on all overall survival (OS), disease-free survival (DFS), relapse-free survival (RFS), disease specific survival (DSS) and progression free interval (PFI) was specific for HCC among other 29 cancer types. Subgroup analysis showed that *ADAMTS5* overexpression was significantly associated with poorer OS in patients with HCC. Finally, *ADAMTS5* might participate in the status conversion from metabolic-dominant to extracellular matrix-dominant, and the activation of ECM-related biological process might contribute to high higher mortality risk for patients with HCC. **Conclusion:** *ADAMTS5* may play an important role in the progression of HCC, and may be considered as a novel and effective biomarker for predicting prognosis for patients with HCC.

Keywords

ADAMTS5, hepatocellular carcinoma, TCGA, GEO, prognostic biomarker, ECM, contradictory role

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Introduction

HCC, accounting for 75-85% cases of liver cancer, is considered as the sixth common occurring cancer and the fourth most common cause of cancer-related death worldwide,[1] especially in East Asia.[2] HCC is characterized by high invasiveness, high metastasis potential, high rate of recurrence and low survival rate. In the past 2 decades, death rates associated with HCC still continue to increase. Additionally, multiple predisposing parameters are known to contribute to the development of HCC, the most common of those being chronic viral hepatitis (B and C), alcohol abuse, and exposure to aflatoxins,[3] and it is predicted that the incidence of HCC will go to the peak in 2030.[4] Hence, effective prognostic biomarkers and novel therapeutic targets are urgently required.

ADAMTS5 belongs to the *ADAMTS* family, which participated in normal biological as well as pathological processes, such as cancers.[5] Recently, increasing evidence indicated the potential role of *ADAMTS5* in multiple types of cancers, overexpression of *ADAMTS5* promote the development of human glioblastomas,[6] and downregulation or hypermethylation of *ADAMTS5* is reported to inhibite many kinds of cancers,

[1] Department of Gastrointestinal Surgery, Xiamen Cancer Center, The First Affiliated Hospital of Xiamen University, Xiamen, Fujian, People's Republic of China
[2] Department of Clinical Medicine, Fujian Medical University, Fuzhou, Fujian, People's Republic of China

Corresponding Author:
Zhengjie Huang, Department of Gastrointestinal Surgery, Xiamen Cancer Center of The First Affiliated Hospital of Xiamen University, 55 Zhen Hai Road, Si Ming District, Xiamen 361003, Fujian Province, People's Republic of China.
Email: huangzhengjie@xmu.edu.cn

including human colorectal cancer,[7] gastric cancer,[8] melanoma.[9] To date, few study reported the association between HCC and *ADAMTS5*, Chongyi Li et al[10] have reported low expression of *ADAMTS5* protein associates with progression and poor prognosis of HCC. However, multiple online websites showed high *ADAMTS5* expression was an independent risk factor for HCC development, thus the exact role of *ADAMTS5* in HCC is unclear and contradictory. In the study, we performed a systemic analysis to reevaluate prognostic value and revealed molecular mechanisms in HCC.

Materials and Methods

Three gene expression profiles(GSE14520, GSE36376, GSE76427) were downloaded from GEO database (http://www.ncbi.nlm.nih.gov/geo/), the clinical information of GSE14520 was also identified form GEO database, the detailed clinical information was shown in Table 1. GSE14520 contained 219 HCC tumor samples and 241 nontumor samples, GSE36376 contained 240 HCC tumor samples and 193 adjacent non-tumor samples, GSE76427 contained 52 HCC tumor samples and 145 adjacent non-tumor samples. The gene expression profile contained 415 candidates (365 patients and 50 normal candidates) was downloaded from TCGA (https://cancergenome.nih.gov/).

ADAMTS5 mRNA Expression Analysis

ADAMTS5 mRNA was compared between tumor and non-tumor samples by performing R Limma package. Furthermore, *ADAMTS5* mRNA was investigated by analyzing GSE14520 and TCGA among different TNM stages, Tumor Size, CLIP stage, T.

The samples were divided into high- and low- *ADAMTS5* expression groups according to median *ADAMTS5* mRNA level, Kaplan-Meier analysis for OS and DFS was performed to compare the survival rate by analyzing GSE14520 and TCGA dataset. Univariate and multivariate logistic-regression analysis were performed to investigate whether *ADAMTS5* could be independent of other clinical parameters. Besides, the association between *ADAMTS5* expression and OS, DFS, DRF, PFS, DSS, progression free survival(PFS) was analyzed by data mining in Kaplan-Meier plotter (http://kmplot.com/analysis/), GEPIA(http://gepia.cancer-pku.cn/index.html) and Oncolnc (http://www.oncolnc.org/). Additionally, the Kaplan Meier plotter (http://kmplot.com/analysis/) was used for subgroup survival analysis for OS in HCC patients. Furthermore, the association between *ADAMTS5* expression and OS, DFS was studied across 29 cancer types to evaluate the differences related to cancer type by using GEPIA. Finally, the association between *ADAMTS5* mRNA and OS, DSS, PFI was studied across 29 cancer types to evaluate the differences related to cancer type by using TCGA dataset.

The samples were divided into high- and low- *ADAMTS5* expression group according to median *ADAMTS5* mRNA level.

Table 1. Correlation of ADAMTS5 Expression With Clinicopathological Parameters.

Characteristics	No. of patients	ADAMTS5 expression (%)		*P* value
		Low	High	
Age				
<60	176	83	93	0.066
>=60	43	27	16	
Gender				
Female	28	18	10	0.111
Male	191	92	99	
Liver cirrhosis				
No	18	16	2	0.001
Yes	201	94	107	
Multinodular				
No	172	95	77	0.005
Yes	47	15	32	
Main Tumor Size				
<=5	142	78	64	0.0059
>5	77	32	45	
HBV viral status				
No	6	5	1	0.086
Yes	213	105	108	
ALT				
<=50U/L	129	75	54	0.005
>50	90	35	55	
AFP				
<=300ng/ml	121	65	56	0.251
>300ng/ml	98	45	53	
Early recurrence				
No	95	55	40	0.047
Yes	124	55	69	
CLIP stage				
0	96	58	38	0.001
1	75	37	38	
2	35	10	25	
3	13	5	8	
TNM stage				
StageI	91	59	32	0.000367
StageII	78	36	42	
StageIII	49	15	34	

DEGs between high- and low- *ADAMTS5* expression group from GSE14520 were identified using the Limma version 3.36.2 R package, with corrected *P*-value < 0.05 and absolute log fold change (FC) > 0.5 being considered as the cutoff criterion. GO biological process enrichment analysis was performed for up-DEGs and down-DEGs. Besides, co-expression analysis also was performed to identified significant genes associated with *ADAMTS5* by mining TCGA dataset, with *P*-value < 0.001 and spearman correlation coefficient r > 0.6. Finally, GSEA (http://software.broadinstitute.org/gsea/index.jsp) was conducted to investigate pathways and groups of genes that may be associated with *ADAMTS5*, NOM *P*-value < 0.05 was considered significantly enriched.

To furtherly find the gene modules associated with *ADAMTS5*, The samples were divided into high- and low-*ADAMTS5* expression group according to median *ADAMTS5*

Table 2. Logistic Regression of ADAMTS5 Expression Association With Clinical Features.

Clinical characteristics	Number	Odds ratio in ADAMTS5 expression	*P* value
Age(<60 vs >=60)	176 vs 43	0.97(0.94-1.00)	0.051
Gender(Male vs Female)	191 vs 56	0.51(0.21-1.15)	0.11
AFP(<=300ng/ml vs >300ng/ml)	121 vs 196	1.36(0.80-2.33)	0.25
ALT(<=50U/L vs >50U/L)	129 vs 90	2.18(1.26-3.80)	0.005
Cirrhosis(No vs Yes)	18 vs 402	9.10(2.50-58.5)	0.003
HBV viral status(No vs Yes)	6 vs 426	5.14(0.81-99.4)	0.13
Multinodular(No vs Yes)	172 vs 47	2.63(1.34-5.33)	0.005
Main Tumor Size(<=5 vs >5)	142 vs 77	2.18(1.26-3.80)	0.005
Early recurrence(No vs Yes)	97 vs 124	1.71(1.00-2.94)	0.049
CLIP stage(0-1 vs 2-3)	173 vs 96	2.74(1.41-5.54)	0.003
TNM stage(I vs II-III)	93 vs 128	2.53(1.47-4.42)	0.0009

mRNA level, high and low *ADAMTS5* group were considered as external sample traits. Gene co-expression network was constructed to explore the modules highly associated with sample traits, 9 was selected as the soft thresholding power to produce a weighted network. The enrolled genes were clustered into 6 modules except the gray module, the modules with high correlation coeficient were considered candidates relevant to traits, which were picked out to perform further pathway enrichment analysis using an online-based web tool "Metascape" (http://metascape.org/).

Result

The Association of ADAMTS5 Expression With Clinicopathological Parameters

As showed in Table 1, we found the *ADAMTS5* mRNA has a high association with liver cirrhosis (P value = 0.001), main tumor size (P value = 0.0059), multinodular (P value = 0.005), ALT (P = 0.005), early recurrence (P value = 0.047), CLIP stage (P value = 0.001), TNM stage (P value = 0.001). However, there was no significant correlation between age (P value = 0.066), gender (P value = 0.111), AFP (P value = 0.251), HBV viral status (P value = 0.086). Logistic-regression analysis were performed to assess association between *ADAMTS5* mRNA and clinicopathological parameters, which revealed the similar result, the *ADAMTS5* mRNA has a higher association with liver cirrhosis (P value = 0.003), main tumor Size (P value = 0.005), multinodular (P value = 0.005), ALT (P value = 0.005), early recurrence (P value = 0.049), CLIP stage (P value = 0.003), TNM stage (P value = 0.0009), and there was no significant correlation between age (P value = 0.051), gender (P value = 0.11), AFP (P value = 0.25), HBV viral status (P value = 0.13)(Table 2).

The Expression of ADAMTS5 Was Positively Correlated With the Development of HCC

As showed in Figure 1, *ADAMTS5* mRNA was significant higher in HCC tumor samples than normal samples in TCGA (Figure 1AC), GSE14520 (Figure 1D), GSE36376 (Figure 1E) and GSE46427 (Figure 1F), and *ADAMTS5* mRNA was significant higher in HCC tumor than adjacent peritumoral tissues (Figure 1B). Next, we analyzed the *ADAMTS5* mRNA in HCC specimens from different tumor TNM stages, T, main tumor size, CLIP stage in the TCGA and GSE14520 database. In GSE14520, *ADAMTS5* mRNA was significant higher in main tumor size >5 cm than main tumor size <=5cm (Figure 1G), as the CLIP stage (Figure 1H) and TNM stage (Figure 1I) increased, the *ADAMTS5* mRNA increased with statistical significance. In TCGA dataset, as the T (Figure 2J) and TNM stage (Figure 2K) elevated, the *ADAMTS5* mRNA increased with statistical significance. GEPIA also indicated that *ADAMTS5* mRNA had an elevated trend with TNM stage increased (Figure 1L), while the P value = 0.0977.

As showed in Figure 3, HCC patients with high *ADAMTS5* expression were associated with poorer OS(log-rank p = 0.003) in GSE14520(Figure 3A) and poorer OS(log-rank P value < 0.001) in TCGA(Figure 3B). With the increasing *ADAMTS5* mRNA, patients have a worse OS in GSE14520 (Figure 3C) and worse OS(log-rank P < 0.001) in TCGA (Figure 3D). Univariate Cox regression analysis and multivariate Cox regression analysis also displayed *ADAMTS5* was a powerful and independent factor in GSE14520 (Figure 3E, 3G) and TCGA dataset (Figure 3F, 3H). Besides, HCC patients with high *ADAMTS5* mRNA expression were associated with worse DFS (log-rank P value = 0.006) in GSE14520 (Figure 2A), and worse DFS(log-rank P value = 0.023) in TCGA (Figure 2B). Additionally, high *ADAMTS5* mRNA significantly contributed to worse DSS(log-rank P value = 0.025, Figure 2C) and PFI(log-rank P value = 0.006, Figure 2D) in TCGA dataset. Next, we validated the prognosis value in online website, Oncolnc website showed that HCC patients with high *ADAMTS5* mRNA expression were associated with worse OS(log-rank P value = 6.5e-5, Figure 2E); GEPIA website indicated HCC patients with high *ADAMTS5* mRNA expression were associated with worse OS(log-rank P value = 9.8e-5, Figure 2F) and DFS(log-rank P value = 0.0069, Figure 2G); Kaplan Meier plotter indicated high *ADAMTS5* mRNA expression significantly contributed to worse OS(log-rank P value = 1e-6, Figure 2H), relapse-free survival (RFS, log-rank P value = 0.0044, Figure 2I), DSS(log-rank P value = 0.00029, Figure 2J) and progression free survival (PFS, log-rank P value = 0.00014, Figure 2K).

Furthermore, we perform the survival analysis stratified by clinical variables(main tumor size, CLIP stage, TNM stage) in GSE14520 and by clinical covariates(T, TNM stage) in TCGA dataset. Stratification analysis indicated that high *ADAMTS5* expressing HCC patients showed significantly worse OS rate than low *ADAMTS5* expressing HCC patients in all the subgroups (Figure 4). Meantime, we make a stratification analysis by using online website Kaplan Meier plotter, high *ADAMTS5*

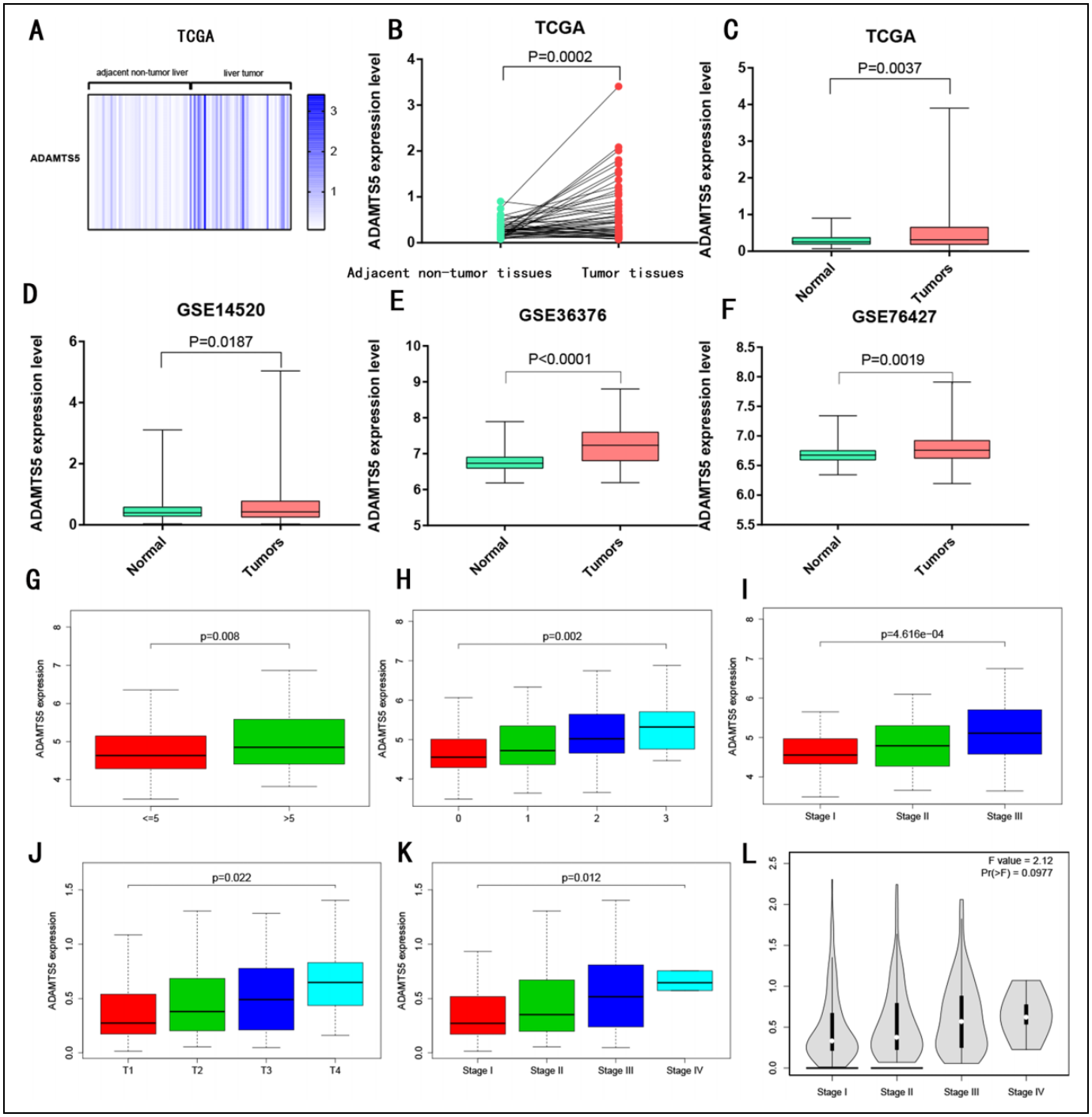

Figure 1. Expression level of ADAMTS5. MRNA expression level of ADAMTS5 between normal samples and HCC tumor samples in (A, C) TCGA; (D) GSE14520; (E) GSE36376; (F) GSE76427. MRNA expression level of ADAMTS5 between adjacent peritumoral tissues and HCC tumor tissues in (B) TCGA. MRNA expression level of ADAMTS5 based on different pathological parameters in (G, H, I) GSE14520; (J, K) TCGA and (L) GEPIA. (G) Main tumor size; (H) CLIP stage; (I, K, L) TNM stage.

expressing HCC patients belonging to male, female, vascular invasion- Micro, vascular invasion- None, hepatitis virus infection-positive, hepatitis virus infection-negative, stage I-II, stage III-IV, stage I, stage II and stage III groups showed significantly worse OS rate than those with low *ADAMTS5* expressing HCC patients (Figure 5).

The prognostic effect of *ADAMTS5* in other 29 cancer types was investigated using GEPIA. As summarized in Table 3, HCC patients in high *ADAMTS5* expression group were associated with worse OS(log-rank P value = 9.80E-05) and DFS(log-rank P value = 0.0069), the impact of *ADAMTS5* on both OS and DFS was specific for HCC only. Next, we investigated the

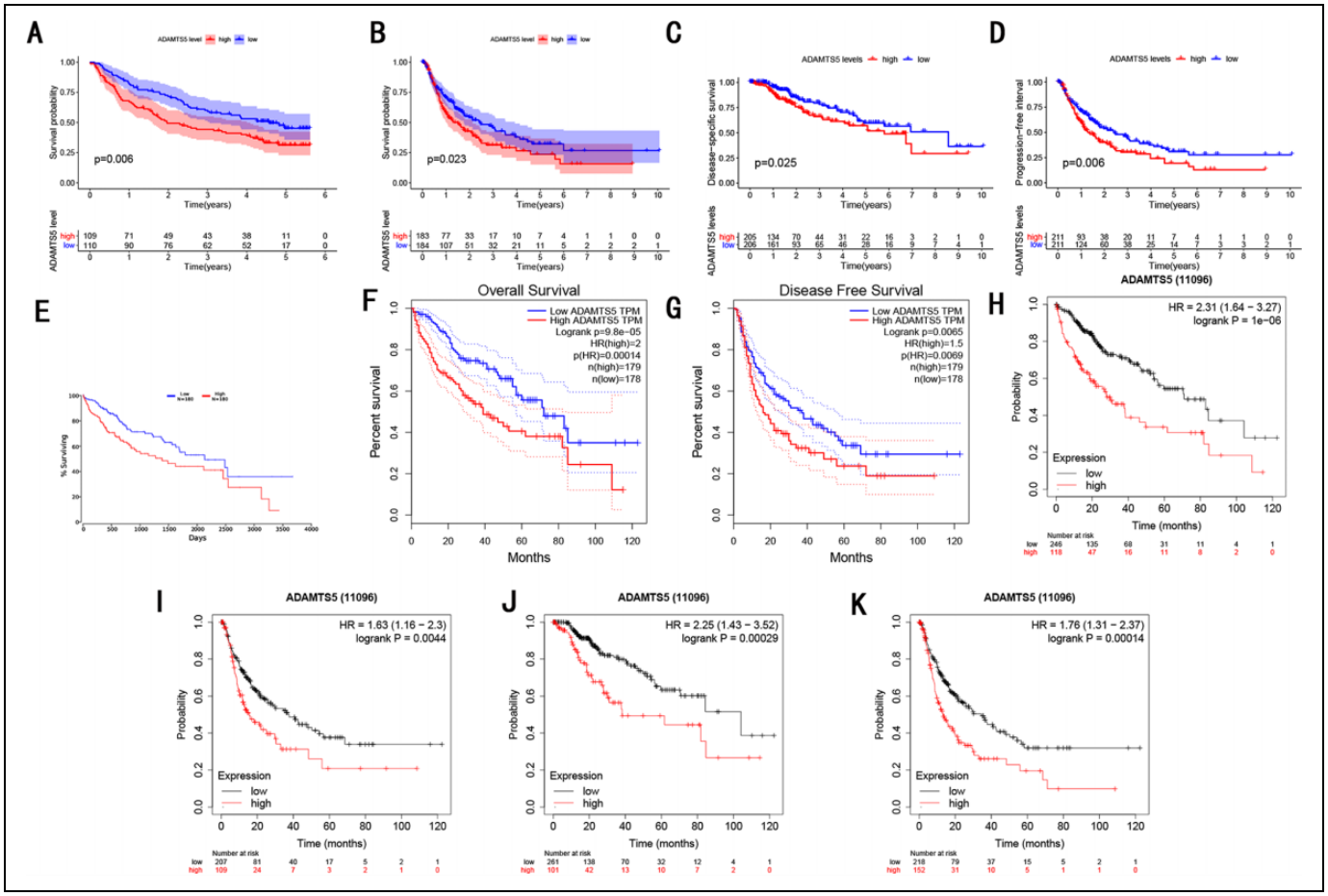

Figure 2. Kaplan-Meier survival analysis based on (A) GSE14520; (B-D) TCGA; (E) oncolnc; (F-G) GEPIA; (H-K) Kaplan-Meier plotter. (A, B, G, I) DFS; (C, J) DSS; (D) PFI; (E, F, H)OS; (K) PFS.

prognostic effect of *ADAMTS5* in other 29 cancer types using TCGA dataset, HCC patients with high *ADAMTS5* mRNA expression were associated with worse OS(log-rank P value = 0.000536), OS(Cox proportional hazards P-value = 2.47E-06), DSS(Cox proportional hazards P value = 0.001772), PFI(log-rank P value = 0.004402), the impact of *ADAMTS5* on all OS, DSS and PFI was specific for HCC only (Table 4).

ADAMTS5 Expression Correlates With Extracellular Matrix (ECM) and Metabolic and Energy Regulation

HCC patients were divided into high- and low- *ADAMTS5* expression group according to median *ADAMTS5* expression level. We identified 89 up-upregulated DEGs and 140 down-regulated DEGs between high and low expression group (Figure 6A). Go enrichment analysis revealed that up-regulated genes were mainly involved in extracellular matrix organization, extracellular structure organization and extracellular matrix disassembly (Figure 6B), down-regulated genes were mainly involved in organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process and alpha−amino acid metabolic process (Figure 6C). To further evaluate the function of *ADAMTS5*, we identified significantly co-expressed genes in HCC using TCGA dataset (P value < 0.0001, Figure 6D). A list of co-expressed genes including Spearman correlation coefficients 0.6 was shown in Table 5, Go enrichment analysis revealed that co-expressed genes were mainly involved in extracellular matrix organization, extracellular structure organization and cell−matrix adhesion (Figure 6E). In order to further explore the pathways regulated by *ADAMTS5* in patients with HCC, we conducted GSEA between high and low *ADAMTS5* expression group to identify the significant pathways (NOM P-value < 0.05), the genes in high *ADAMTS5* expression group were mainly enriched in extracellular matrix (ECM), including KEGG_ECM_RECEPTOR_INTERACTION, KEGG_FOCAL_ADHESION, KEGG_GAP_JUNCTION, KEGG_PATHWAYS_IN_CANCER, KEGG_REGULATION_OF_ACTIN_CYTOSKELETON. As to *ADAMTS5* low-expression group, the genes were enriched in metabolic pathways, such as KEGG_BUTANOATE_METABOLISM, KEGG_FATTY_ACID_METABOLISM, KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM, KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS (Figure 6F).

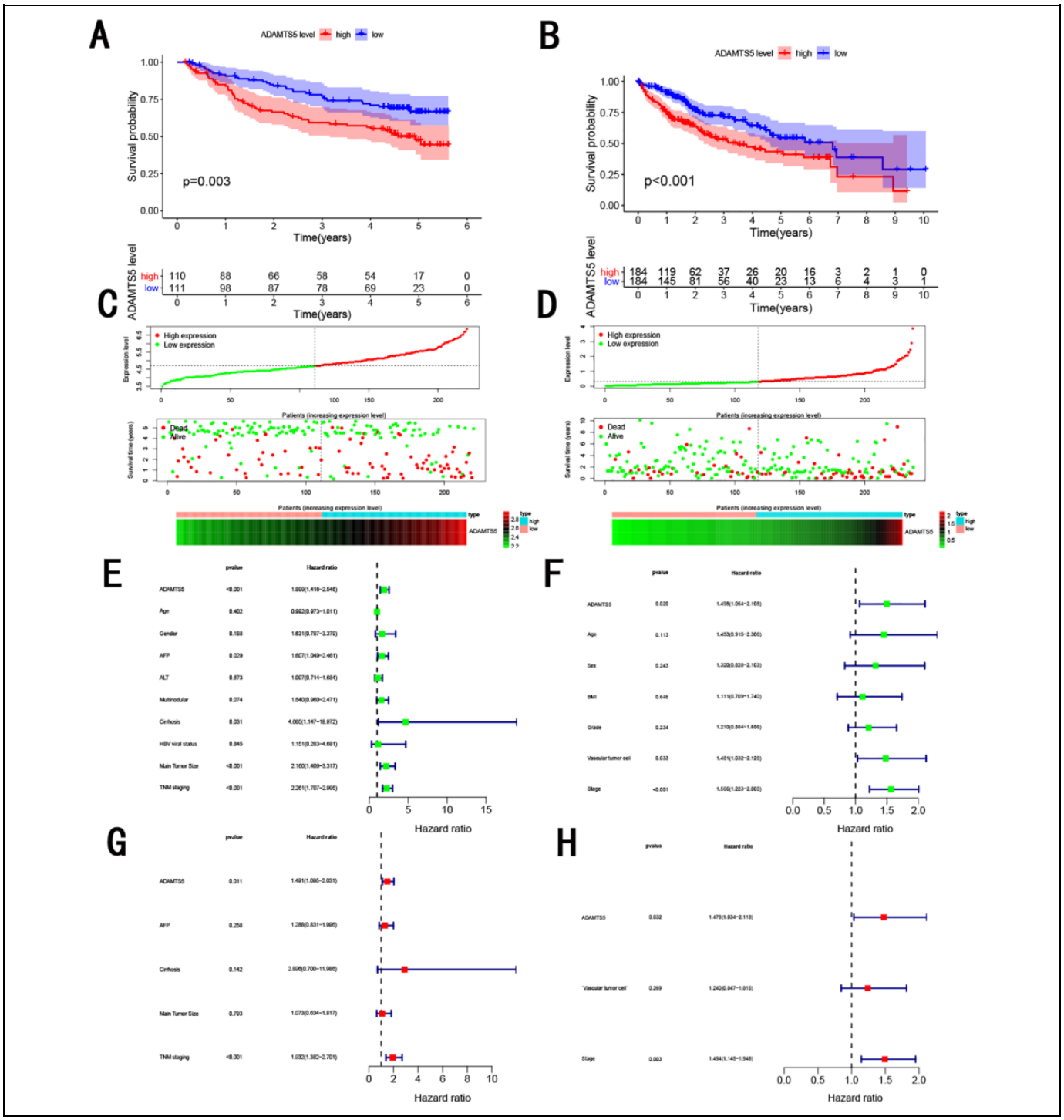

Figure 3. Survival analysis for OS in (A, C, E, G) GSE14520 and (B, D, F, H) TCGA. (A, B) Kaplan-Meier curves of OS; (C, D) distribution of patient survival durations, gene expression levels; (E, F) prognostic value detection of the ADAMTS5 via univariate survival-related analysis; (G, H) prognostic value detection of the ADAMTS5 via multivariate survival-related analysis.

Extracellular Matrix (ECM) Related Process Were Associated With ADAMTS5

To furtherly investigate the potential mechanisms of *ADAMTS5* in HCC, WGCNA was performed to cluster genes that highly correlated with the high *ADAMTS5* group and low *ADAMTS5* group based on the expression profile of GSE14520. We identified 6 modules (Figure 7A) in the study(-excluding a gray module). As showed in Figure 7B, we found genes in green module was highly positively associated with

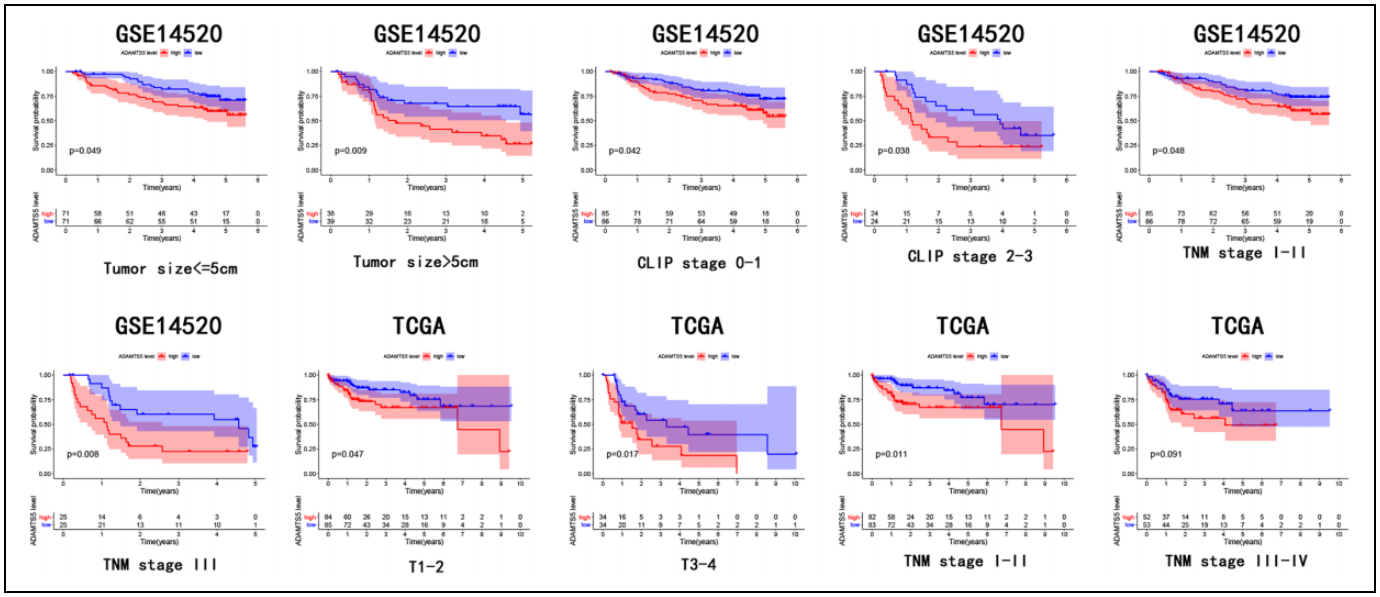

Figure 4. Kaplan–Meier survival analysis of OS based classifier in subgroups of patients with pathological factors in GSE14520 and TCGA.

high *ADAMTS5* group($7x10^{-5}$) and genes in turquoise module was highly positively associated with low *ADAMTS5* group($1x10^{-6}$). Go enrichment analysis was performed to explore the potential mechanisms of genes in green module and turquoise module using online-based web tool "Metascape" (http://metascape.org/), as showed in Figure 7C and Figure 7D, genes in the turquoise module were significantly enriched in metabolic process, such as monocarboxylic acid metabolic process, small molecule catabolic process, and carboxylic acid biosynthetic process. As showed in Figure 7E and Figure 7F, genes in the green module were significantly enriched in extracellular matrix (ECM), such as extracellular matrix organization, response to growth factor, and blood vessel development. Thus *ADAMTS5* might participate in the status conversion from metabolic-dominant to extracellular matrix-dominant, and the activation of ECM-related biological process may might contribute to high higher mortality risk for patients with HCC.

Discussion

ADAMTS5 has been reported to be associated multiple types of cancers with different role,[7,11-13] which may functioned as anti-tumorigenic or pro-tumor role. However, the role of ADAMTS5 is still unclear in HCC, previous study has revealed that high ADAMTS5-expressed HCC had better OS than the patients without or with low expression of ADAMTS5, and the multivariate analysis indicated that ADAMTS5 expression was an independent prognostic factor for HCC patients. Our study demonstrated the opposite result, high ADAMTS5-expressed HCC had poorer OS than patients with low expression, ADAMTS5 expression is also associated with the development of HCC and is an independent prognostic factor for HCC patients. Moreover, the impact of ADAMTS5 expression on OS, DFS, RFS, DSS and PFI was specific for HCC among other 29 cancer types, which indicated the unique prognostic role of ADAMTS5 in HCC.

ECMs are highly specialized 3-D imensional macromolecular networks. which is composed of various proteins, such as collagen, proteoglycans, laminin, and fibronectin.[14] ECMs is dynamic and versatile to regulated cell function through direct or indirect means to regulate cell proliferation, migration, differentiation and invasiveness to maintain tissue homeostasis and functions,[14-17] abnormal dynamics may lead to abnormal behaviors of cells in the local microenvironment through modulating the hallmarks of cancer, including dysregulated cellular metabolism, the evasion of immune destruction, sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis.[18] Thus ECMs is critical for malignancy. During the normal condition, ECMs remodeling is tightly regulated through accumulation of inflammatory mediators, such as growth factors, cytokines and ECM-degrading enzymes, and ECM-degrading enzymes is considered as a crucial factor to control the ECMs remodeling activity, including matrix metalloproteinases (*MMPs*), plasminogen activators, a disintegrin and metalloproteinases (*ADAMs*), *ADAMs* with thrombospondin motifs (*ADAMTS*),[19] and abnormal ECMs remodeling is usually documented in previous studies related with various pathologies and is a crucial factor for initiation or progression of cancer.[15,20] Consistently, abnormal expression of many matrix-degrading enzymes are frequent in many types of human cancers.[21,22] Matrix-degrading enzymes can promote malignancy via various ways including degrade basement membranes, activate growth factors to enhance cell growth, invasion and neovascularization.[23,24] As an important member of matrix-degrading enzymes, *ADAMTS* family has been found to take part in various biological processes, including tissue

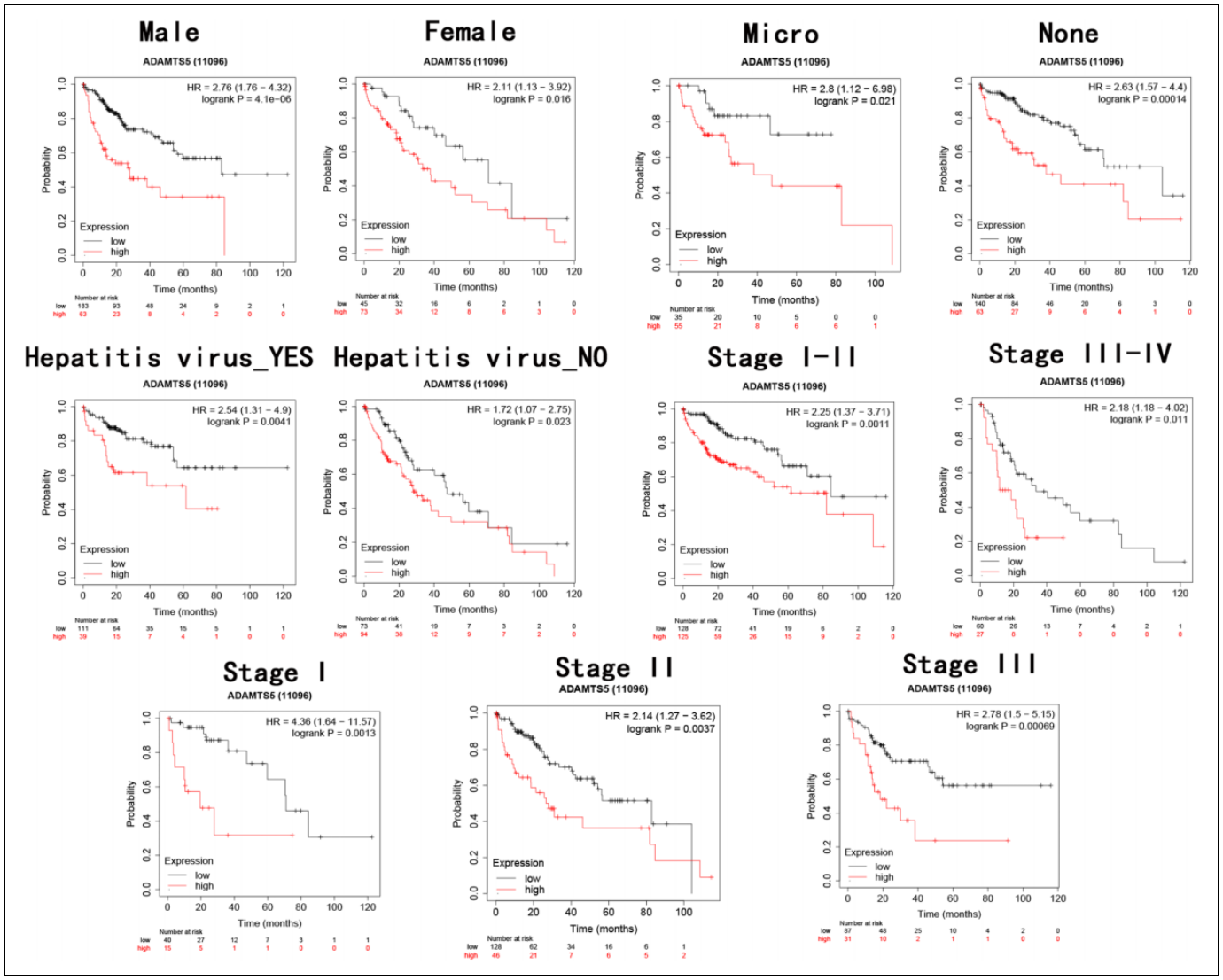

Figure 5. Kaplan–Meier survival analysis of OS based classifier in subgroups of patients with clinical characteristics online website Kaplan-Meier plotter.

morphogenesis, inflammation and angiogenesis.[25] As we know more about *ADAMTS* family, it surprised us that many of them(10 of 19) play an important role in the tumor pathological process, such as *ADAMTS1*, *ADAMTS4*, *ADAMTS5*, *ADAMTS9* and so on. *ADAMTS5* was first found in 1999 and was identified as a key enzyme in osteoarthritis by degrading aggrecan as an aggrecanase.[26] In recently, several studies have reported that aberrant *ADAMTS5* expression in various cancers, including human glioblastomas,[6] human colorectal cancer,[7] human prostate cancer,[27] non-small cell lung cancer,[28] gastric cancer,[8] melanoma.[8] However, the functions of *ADAMTS5* is not the same, they perform as an oncogene in human glioblastomas and non-small cell lung cancer. On the contrary, the *ADAMTS5* functions as a tumor suppressor in breast cancer and human prostate cancer. Hence, *ADAMTS5* may have different roles in different cancer tissues. To date, few study reported the association between HCC and *ADAMTS5*, Chongyi Li et al[10] indicated low *ADAMTS5* expression is related with poor prognosis and functions as an anti-tumorigenic protein, and they reported that *ADAMTS5* expression could downregulate VEGF level to inhibit tumor growth and angiogenesis in HCC cell line, lost expression of *ADAMTS5* protein is associated with poor prognosis of HCC. However, multiple online websites present the opposite result that high *ADAMTS5* expression is associated with poor survival in patients with HCC. Meantime, our result demonstrated that high *ADAMTS5* was associated with poorer survival in HCC patients, and *ADAMTS5* was an independent risk factor for survival in HCC. Our result had also suggested that the impact of *ADAMTS5* on prognosis was specific for HCC. In addition, *ADAMTS5* was associated with various types of survival evaluation index in HCC, including OS, RFS, DFS, DSS, PFI.

The GO enrichment analysis based on DEGs suggested that *ADAMTS5* expression correlates with ECM and metabolic and

Table 3. Survival Analysis Correlations for ADAMTS5 Across 29 Cancer Types From the Online Website (GEPIA).

Tumor type		OS		DFS	
Abbreviation	Detail	HR	ADAMTS5 and overall survival log-rank *P* value	HR	ADAMTS5 and disease-free survival log-rank *P* value
LIHC	Liver hepatocellular carcinoma	2	9.80E-05	1.5	0.0069
ACC	Adrenocortical carcinoma	2.2	0.046	1.8	0.093
BLCA	Bladder Urothelial Carcinoma	1.1	0.47	0.89	0.49
BRCA	Breast invasive carcinoma	0.97	0.87	0.97	0.87
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	1.8	0.017	1.7	0.061
CHOL	Cholangiocarcinoma	0.9	0.83	0.9	0.81
COAD	Colon adenocarcinoma	1.5	0.081	1.9	0.0067
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	0.46	0.29	0.54	0.34
ESCA	Esophageal carcinoma	1.5	0.076	1.2	0.47
GBM	Glioblastoma multiforme	1.3	0.12	1.2	0.45
HNSC	Head and Neck squamous cell carcinoma	1.2	0.25	1.1	0.44
KICH	Kidney Chromophobe	1.8	0.43	1.4	0.63
KIRC	Kidney renal clear cell carcinoma	0.75	0.057	0.96	0.84
LAML	Acute Myeloid Leukemia	0.81	0.47	1	1
LGG	Brain Lower Grade Glioma	0.6	0.006	0.96	0.79
LUAD	Lung adenocarcinoma	1.2	0.15	1.1	0.62
LUSC	Lung squamous cell carcinoma	1.2	0.17	1.1	0.58
OV	Ovarian serous cystadenocarcinoma	0.88	0.3	0.78	0.048
PAAD	Pancreatic adenocarcinoma	1.1	0.7	1.1	0.79
PCPG	Pheochromocytoma and Paraganglioma	4.9	0.11	1.3	0.64
PRAD	Prostate adenocarcinoma	0.53	0.63	0.92	0.68
READ	Rectum adenocarcinoma	0.64	0.36	0.76	0.57
SARC	Sarcoma	0.93	0.73	0.96	0.81
SKCM	Skin Cutaneous Melanoma	1	0.84	1.1	0.43
STAD	Stomach adenocarcinoma	1.3	0.15	0.88	0.52
TGCT	Testicular Germ Cell Tumors	2.1	0.51	1.3	0.44
THCA	Thyroid carcinoma	6.5	0.0045	0.81	0.93
THYM	Thymoma	0.6	0.49	0.85	0.71
UCSC	Uterine Corpus Endometrial Carcinoma	0.97	0.94	0.74	0.35
UCS	Uterine Carcinosarcoma	0.87	0.7	0.8	0.58

energy regulation. The co-expressed genes associated with *ADAMTS5* were mainly involved in extracellular matrix organization, extracellular structure organization and cell−matrix adhesion. Besides, the GSEA enrichment analysis revealed the genes in high *ADAMTS5* expression group were mainly enriched in extracellular matrix (ECM), while the genes in low *ADAMTS5* expression group were enriched in metabolic pathways. Furthermore, WGCNA method was performed to cluster gene module, and found genes in green module was highly positively associated with high *ADAMTS5* group($7x10^{-5}$) and genes in turquoise module was highly positively associated with low *ADAMTS5* group($1x10^{-6}$), GO enrichment analysis revealed genes in the turquoise module were significantly enriched in metabolic pathways, and extracellular matrix. These results implied that *ADAMTS5* might participate in the status conversion from metabolic-dominant to extracellular matrix-dominant, and *ADAMTS5* might affect the ECM biological process and consequently contributed to tumor progression. ECM could mediate microenvironment to regulate the metastasis,[29] it has reported that *ADAMTS* can degrade the ECM to promote invasion and metastasis, including *ADAM-8*, *ADAM-12*, *ADAM-15*, and *ADAM-28* in non-small cell lung cancer,[30-33] *ADAMTS4* and *ADAMTS5* in glioblastomas,[6] *ADAM-9* in liver cancer.[34] Thus, there may be 2 reasons to provide explanation of the contradictory conclusions between the result of Chongyi Li' study and our research, Firstly, tumor microenvironment is a complex macromolecular networks, *ADAMTS5* could participate in tumor microenvironment to influence the various types of hallmarks of cancer, including dysregulated cellular metabolism, the evasion of immune destruction, sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, the previous study solely focus on HCC cells and sole cancer hallmark,[18] which may be different to explain the role of *ADAMTS5* in tumor microenvironment. Secondly, as an important member of matrix-degrading enzymes, *ADAMTS5* may promote HCC via many ways, including degrade basement membranes, activate growth factors to enhance cell growth, invasion and neovascularization.[23,24] It have not

Table 4. Survival Analysis Correlations for ADAMTS5 Across 29 Cancer Types From the Cancer Genome Atlas (TCGA) Project.

Tumor type		OS			DSS		PFI	
		KM Log-Rank	Cox proportional hazards analysis					
Abbreviation	Detail	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
LIHC	Liver hepatocellular carcinoma	0.000536	2.37(1.65,3.39)	2.47E-06	2.08(1.31,3.31)	0.001772	1.59(1.15,2.19)	0.004402
ACC	Adrenocortical carcinoma	0.118575	1.52(0.92,2.48)	0.094979	1.48(0.88,2.46)	0.130936	1.08(0.67,1.75)	0.727289
BLCA	Bladder Urothelial Carcinoma	0.779791	1.21(0.91,1.61)	0.175336	1.44(1.06,1.96)	0.01968	1.16(0.88,1.55)	0.280178
BRCA	Breast invasive carcinoma	0.809903	0.97(0.77,1.22)	0.840395	1.27(1.08,1.50)	0.003761	1.06(0.92,1.22)	0.372714
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.035927	1.25(0.74,2.10)	0.399366	1.29(0.88,1.90)	0.185864	1.27(0.89,1.79)	0.174198
CHOL	Cholangiocarcinoma	0.525155	0.96(0.21,4.33)	0.960122	0.92(0.23,3.56)	0.905325	0.88(0.21,3.59)	0.863125
COAD	Colon adenocarcinoma	0.94546	0.98(0.64,1.48)	0.923755	1.21(0.72,2.01)	0.461237	1.24(0.85,1.81)	0.251106
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	0.314884	0.25(0.02,2.70)	0.25703	0.21(0.01,6.28)	0.375869	0.27(0.03,2.16)	0.22034
ESCA	Esophageal carcinoma	0.346123	1.17(0.78,1.75)	0.438162	1.48(0.96,2.28)	0.069605	1.14(0.81,1.62)	0.433436
GBM	Glioblastoma multiforme	0.222381	1.08(0.72,1.60)	0.693639	1.22(0.75,1.96)	0.4112	0.92(0.61,1.39)	0.720239
HNSC	Head and Neck squamous cell carcinoma	0.158117	1.08(0.84,1.39)	0.529964	0.95(0.69,1.32)	0.789502	0.96(0.74,1.26)	0.817289
KICH	Kidney Chromophobe	0.737972	0.78(0.24,2.49)	0.682603	0.95(0.30,2.92)	0.930898	1.05(0.43,2.55)	0.911317
KIRC	Kidney renal clear cell carcinoma	0.117953	0.79(0.65,0.97)	0.028268	0.85(0.66,1.08)	0.184744	0.91(0.75,1.10)	0.333099
LAML	Acute Myeloid Leukemia	0.123789	1.19(0.64,2.18)	0.572802				
LGG	Brain Lower Grade Glioma	0.038998	0.45(0.28,0.70)	0.00046	0.42(0.26,0.68)	0.000519	0.66(0.46,0.95)	0.02713
LUAD	Lung adenocarcinoma	0.136744	1.10(0.86,1.41)	0.424685	1.11(0.80,1.54)	0.501242	1.24(0.97,1.59)	0.07874
LUSC	Lung squamous cell carcinoma	0.390184	1.10(0.85,1.41)	0.452687	0.94(0.61,1.46)	0.810799	0.94(0.68,1.29)	0.727165
OV	Ovarian serous cystadenocarcinoma	0.335461	0.99(0.80,1.22)	0.963084	0.97(0.78,1.22)	0.848584	0.98(0.80,1.19)	0.870637
PAAD	Pancreatic adenocarcinoma	0.413798	1.39(0.85,2.27)	0.18345	1.53(0.89,2.61)	0.116119	1.04(0.65,1.67)	0.854204
PCPG	Pheochromocytoma and Paraganglioma	0.042833	1.00(0.35,2.83)	0.995274	0.88(0.25,3.03)	0.847106	1.23(0.65,2.31)	0.513304
PRAD	Prostate adenocarcinoma	0.582847	0.53(0.10,2.73)	0.452369	0.08(0.01,3.09)	0.178287	0.50(0.31,0.82)	0.005647
READ	Rectum adenocarcinoma	0.68499	1.61(0.62,4.22)	0.325264	0.76(0.18,3.12)	0.705365	1.09(0.46,2.58)	0.830455
SRAC	Sarcoma	0.576454	0.82(0.63,1.08)	0.16798	0.93(0.82,1.05)	0.285141	0.97(0.78,1.20)	0.786088
SKCM	Skin Cutaneous Melanoma	0.684798	0.952(0.85,1.06)	0.402038	0.92(0.69,1.23)	0.603342	0.93(0.85,1.02)	0.165014
STAD	Stomach adenocarcinoma	0.334916	1.51(1.10,2.07)	0.009727	1.12(0.74,1.68)	0.586906	0.90(0.64,1.27)	0.583443
TGCT	Testicular Germ Cell Tumors	0.46775	1.08(0.23,5.00)	0.917385	0.87(0.13,5.65)	0.88905	0.99(0.64,1.53)	0.992886
THCA	Thyroid carcinoma	0.002857	2.16(1.17,3.97)	0.013331	2.75(1.22,6.17)	0.014279	0.93(0.65,1.34)	0.734213
THYM	Thymoma	0.758544	0.52(0.11,2.44)	0.409842	0.05(0.0003,8.60)	0.260126	0.96(0.56,1.64)	0.884015
UCEC	Uterine Corpus Endometrial Carcinoma	0.084722	1.11(0.84,1.48)	0.44014	1.09(0.82,1.44)	0.544617	1.03(0.84,1.26)	0.75889
UCS	Uterine Carcinosarcoma	0.564656	0.97(0.65,1.45)	0.911449	0.94(0.61,1.44)	0.790456	1.03(0.71,1.52)	0.841463

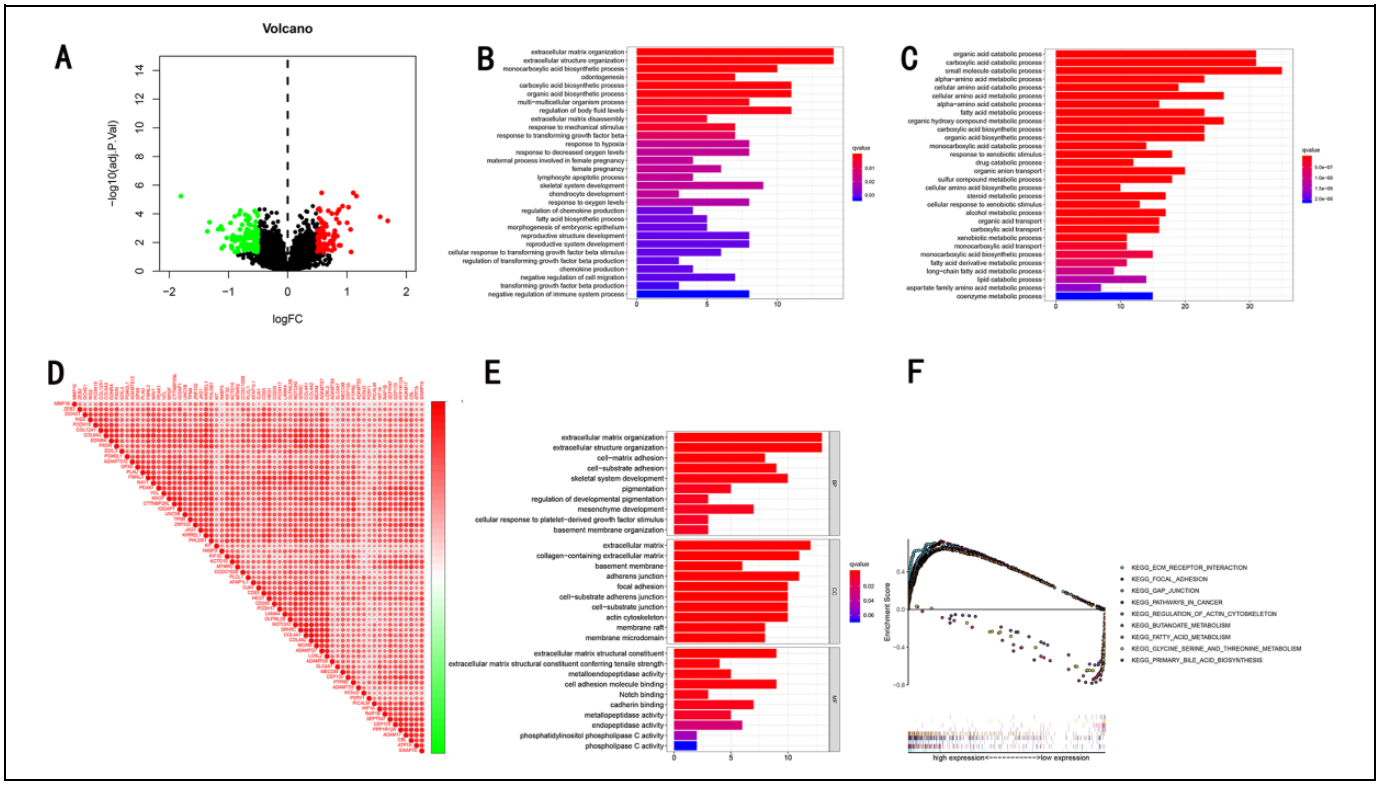

Figure 6. GO enrichment analysis based on DEGs and GSEA enrichment analysis revealed the biological processes and KEGG signaling pathways associated with ADAMTS5 in HCC. (A) Volcano plot of DEGs between high- and lowADAMTS5 expression group. The red points represent high expression genes, the green points represent low expression genes, the black points represent genes with no significant difference (corrected *P* value 0.5). (B) Biological processes associated with upregulated genes. (C) Biological processes associated with down-regulated genes. (D) Co-expressed genes associated with ADAMTS5 in HCC using TCGA ($r > 0.600$, $p < 0.001$). (E) Go enrichment analysis associated with co-expressed genes. (F) GSEA enrichment analysis revealed KEGG signaling pathways which were active in high expression or low expression group.

Table 5. List of Genes Associated With ADAMTS5 Expression ($r > 0.600$, $P < 0.001$).

Gene symbol	Spearman correlation coefficient r	Gene symbol	Spearman correlation coefficient r	Gene symbol	Spearman correlation coefficient r
CD200	0.6	PEAK1	0.617	SPARC	0.64
NOTCH3	0.6	PLAU	0.617	KCNJ2	0.641
SLC4A7	0.601	PICALM	0.618	LAMA4	0.642
ADAMTS9	0.602	ADAMTS12	0.618	IQGAP1	0.647
KIT	0.603	JAG1	0.619	ZNF532	0.648
DCHS1	0.604	CEP170	0.623	HIF1A	0.65
MTMR2	0.604	NAV1	0.624	PPP1R12A	0.651
CEP135	0.605	PLCL1	0.624	PCDH18	0.652
SWAP70	0.607	AFAP1L1	0.624	CD93	0.653
PTPRE	0.607	EDIL3	0.624	COL6A3	0.661
MYOF	0.608	COL12A1	0.626	COL4A1	0.661
ADAM17	0.608	FMNL3	0.628	VCL	0.667
N4BP3	0.609	OLFML2B	0.628	CTTNBP2NL	0.672
GJA1	0.61	GPX8	0.629	PCDH17	0.672
P2RY1	0.612	KIRREL1	0.629	PHLDB1	0.673
ZEB2	0.613	COL4A2	0.631	LOXL2	0.678
HEG1	0.613	RAP1B	0.633	UNC5B	0.685
TPM4	0.614	NID2	0.633	PXDN	0.691
KIF3C	0.614	SEPTIN7	0.634	PGM2L1	0.691
MMP16	0.614	KCTD10	0.635	EDNRA	0.691
CCDC102B	0.615	CBL	0.636	ADAMTS7	0.693
ATP7A	0.616	MECOM	0.637	MCAM	0.701

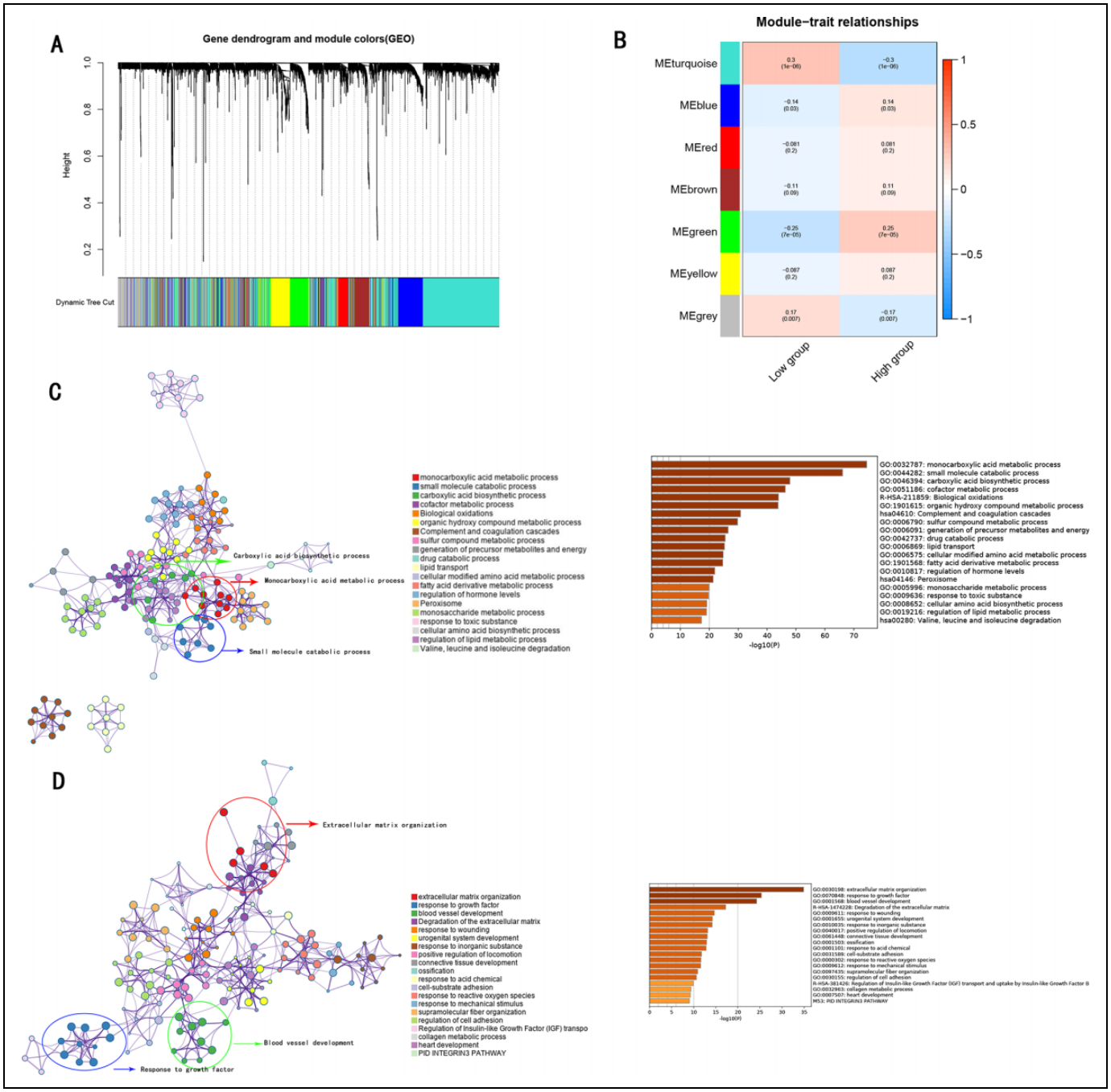

Figure 7. WGCNA predicted biological pathways associated with ADAMTS5. (A) The cluster dendrogram of coexpression network modules. (B) The gene clusters obtained by WGCNA method. (C) Significantly enriched biological process of the co-expressed genes in turquoise module. (D) Significantly enriched biological process of the co-expressed genes in green module.

clearly determined the mechanisms might regulate the effects of *ADAMTS5* on HCC, and further functional experiments are necessary to reveal the elusive mechanisms of *ADAMTS5* in HCC. Considered of the controversial results, it is suggested meta-analysis to evaluate the association between *ADAMTS5* and HCC. Finally, almost all the evidence comes from bulk tissue RNA-seq data, which indicated *ADAMTS5* expression level could be influenced both by cancer cells and surrounding tumor microenvironment in our study, further search should be carried to the release the clear role of *ADAMTS5* in HCC. Even though, we could cautiously postulate that *ADAMTS5* is a novel biomarker predicting unfavorable prognosis for HCC.

Conclusion

To conclude, Our data suggested that *ADAMTS5* is a prognosis-related gene for HCC, and the impact of *ADAMTS5* on all survival was specific for HCC only. With a potential role in

ECM and metabolic and energy regulation. *ADAMTS5* is a promising therapeutic target for patients with HCC when taking subsite into consideration.

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Declaration of Conflicting Interests

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ORCID iD

Zhengjie Huang https://orcid.org/0000-0001-6298-8112

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