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# Angiogenic Factor with G Patch and FHA Domains 1 (AGGF1) Acts as Diagnostic Biomarker and Adverse Prognostic Factor of Hepatocellular Carcinoma (HCC): Evidence from Bioinformatic Analysis

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Data Collection B  
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**Background:** Angiogenic factor with G patch and FHA domains 1 (AGGF1) is a novel identified initiator of angiogenesis through promoting the proliferation of endothelial cells. The continuous angiogenesis plays a key role in the growth, invasion, and metastasis of hepatocellular carcinoma (HCC), while the diagnostic and prognostic roles of AGGF1 for HCC need to be further studied.

**Material/Methods:** The mRNA sequencing datasets and clinical features of HCC patients were extracted from The Cancer Genome Atlas database. The relationship between clinical features and AGGF1 expression was analyzed by Wilcoxon test. Further validation explorations were carried out using online database Oncomine. The diagnostic receiver operating characteristic curves of AGGF1 and alpha-fetoprotein were compared to examine the diagnostic efficacy of AGGF1. Survival analysis and Gene Set Enrichment Analysis were performed to explore the prediction value and potential mechanism of AGGF1 dysregulation in HCC.

**Results:** Comprehensive overexpression of AGGF1 was observed in HCC, correlating with poor overall survival. Upregulated level of AGGF1 was statistically associated with poor differentiated histological grade, advanced cancer stage and T classification. AGGF1 was a more effective diagnostic marker than alpha-fetoprotein in HCC. Several important pathways related to HCC including pathway in cancer and P53 signaling pathway were differentially enriched in the high AGGF1 expression phenotype.

**Conclusions:** AGGF1 was a potential diagnostic and prognostic marker for poor clinical outcomes in HCC patients. Moreover, vital pathways regulated by AGGF1 in HCC may include regulation of autophagy, Wnt signaling pathway, pathway in cancer, cell cycle, and P53 signaling pathway.

**MeSH Keywords:** **Biological Markers • Carcinoma, Hepatocellular • Prognosis**

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## Background

Liver cancer accounts for a sixth of new neoplasm cases and a third of cancer-related mortality cases worldwide, with estimated 841 000 cases diagnosed and 781 000 deaths in 2018 [1]. Hepatocellular carcinoma (HCC), which usually occurs in patients with chronic liver disease, is the most predominant pathological type of liver cancer. HCC is usually diagnosed at the advanced stage; the 5-year overall survival (OS) rate of HCC is 10% for locally advanced and 3% for metastatic, respectively [2].

Angiogenesis plays a critical role in cancer growth and progression. As a hyper-vascularized tumor, the aggressive and metastatic features of HCC lead to poor clinical outcomes [2]. Angiogenic factor with G-patch and FHA domain 1 (AGGF1) is a novel pro-angiogenic factor that was initially characterized in Klippel-Trenaunay syndrome [3]. A previous study showed that AGGF1 was upregulated in HCC [4], while its potential roles as diagnostic and prognostic marker for HCC still need to be elucidated.

In this current study, we utilized the The Cancer Genome Atlas (TCGA) database to compare AGGF1 mRNA expression between HCC tumor tissues and adjacent tissues. We also attempted to explore the relationship between the expression of AGGF1 mRNA and clinical features and OS of HCC patients. Subsequently, we verified the credibility in Oncomine (<https://www.oncomine.org/>). Furthermore, Gene Set Enrichment Analysis (GSEA) was performed to identify the signaling pathways which would be involved in HCC.

## Material and Methods

### Collection of RNA-sequencing data and bioinformatics analysis

We firstly explored the expression and distribution of AGGF1 among different cancer tissues using GEPIA database (<http://gepia.cancer-pku.cn/>) [5]. To create the data set, transcriptome profiling of The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) including 374 tumor and 50 adjacent normal samples were downloaded from the webserver (<https://portal.gdc.cancer.gov/>, Workflow Type: HTSeq-FPKM, accessed on July 20, 2019). The corresponding survival and clinic profiles were also obtained from TCGA Data Portal with a total of 377 HCC patients. The clinical and AGGF1 mRNA expression matrix information were matched by sample ID. Oncomine is an online genome-wide expression cancer microarray database [6]. In order to further verify the TCGA analysis results, we conducted Oncomine exploration.

**Table 1.** The Clinical Characteristics of HCC Patients from TCGA.

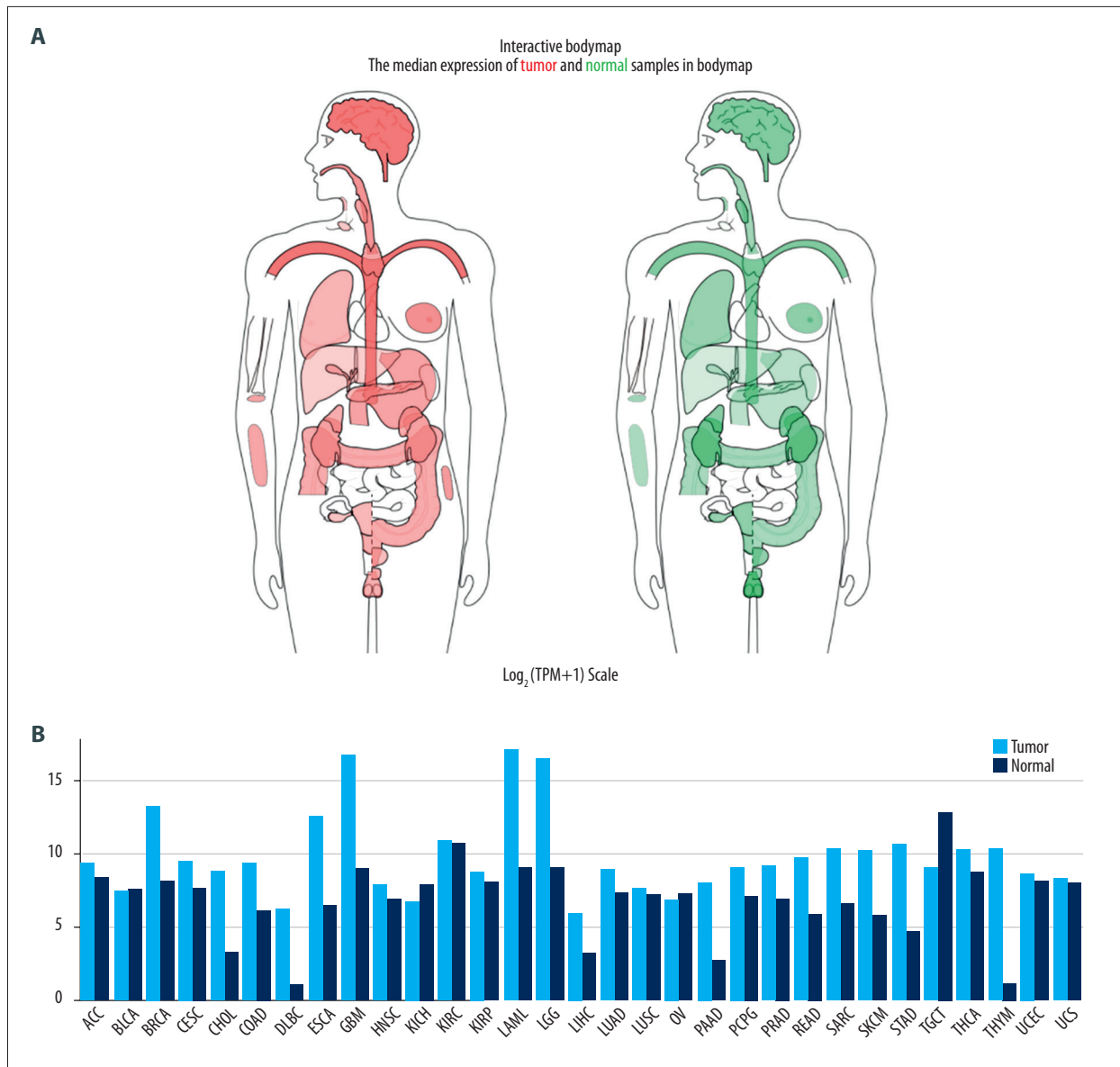
Clinical characteristics		Total (%) N=377	
Age(y)	≤60	180	(47.7)
	>60	196	(52.0)
	Unavailable	1	(0.3)
Gender	Female	122	(32.4)
	Male	255	(67.6)
Follow-up state	Living	249	(66.0)
	Dead	128	(34.0)
Grade	G1	55	(14.6)
	G2	180	(47.7)
	G3	124	(32.9)
	G4	13	(3.4)
	Unavailable	5	(1.3)
Stage	I	175	(46.4)
	II	87	(23.1)
	III	86	(22.8)
	IV	5	(1.3)
	Unavailable	24	(6.4)
T	T1	185	(49.1)
	T2	95	(25.2)
	T3	81	(21.5)
	T4	13	(3.4)
	TX	1	(0.3)
	Unavailable	2	(0.5)
N	N0	257	(68.2)
	N1	4	(1.1)
	NX	115	(30.5)
	Unavailable	1	(0.3)
M	M0	272	(72.1)
	M1	4	(1.1)
	MX	101	(26.8)

HCC – hepatocellular carcinoma; TCGA – The Cancer Genome Atlas.

Due to the datasets used in our study were public and available online, Ethical approval and informed consent are not required.

### Gene Set Enrichment Analysis (GSEA)

In our study, the genomic expression profiles of 374 HCC samples were classified into high (n=187) and low (n=187) subgroups based on the median value of AGGF1 mRNA expression. GSEA, a computational approach determines statistically significant, concordant differences of a priori defined set of



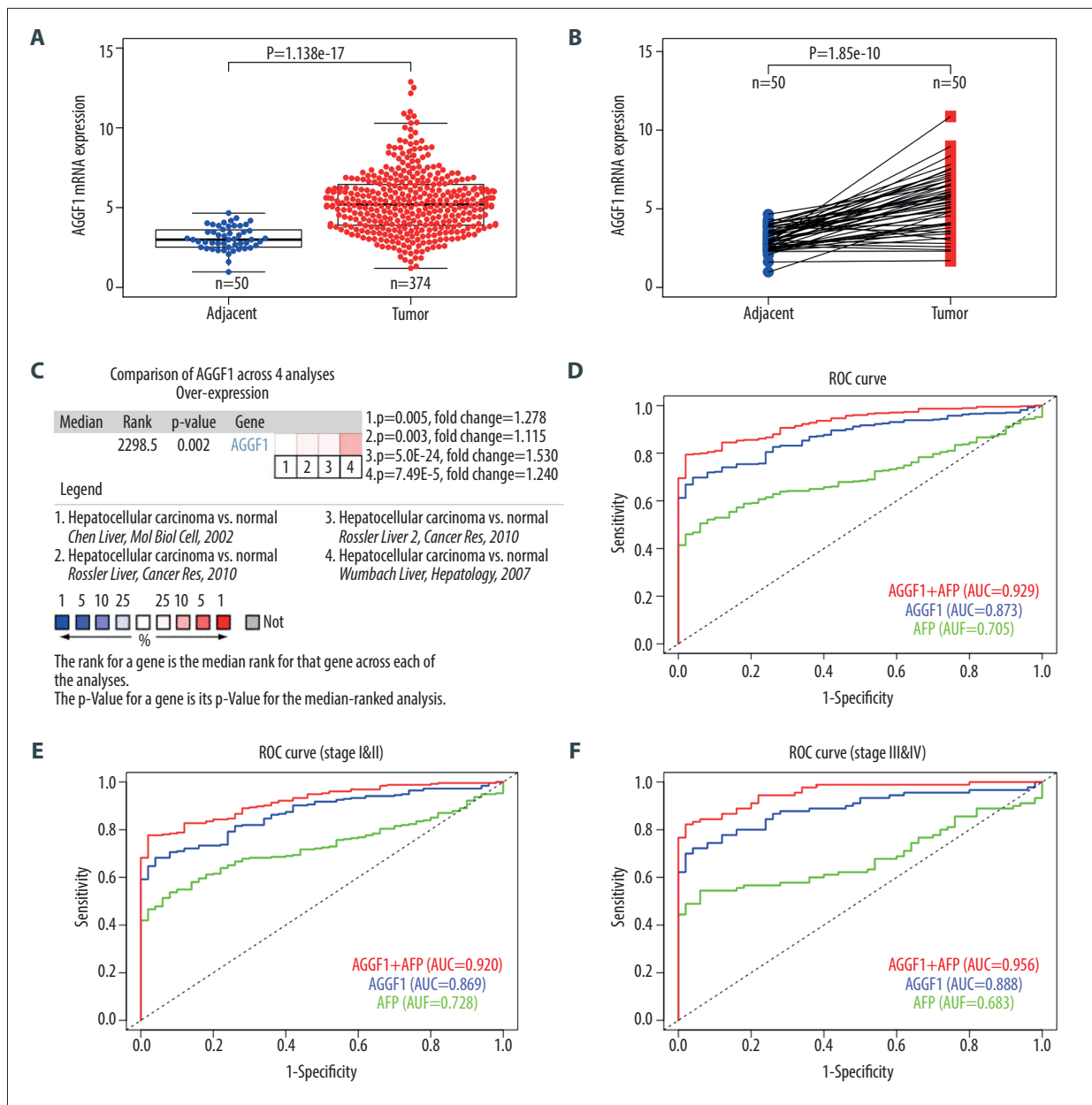
**Figure 1.** AGGF1 mRNA expression in body map (A) and different levels in tissues (B). The height of the column represents the median expression in tumor or normal tissues. AGGF1 – angiogenic factor with G patch and FHA domains 1.

genes, was carried out between the 2 subgroups [7]. The version of GSEA software was v3.0. Gene set permutations were performed 1000 times for each analysis. Enrichment results with a *P*-value <0.05 and false discovery rate (FDR) <0.25 were considered statistically significant.

### Statistical analysis

The statistical analyses and plots were conducted using R (version 3.5.2) and SPSS (version 23.0) software. Differences in AGGF1 mRNA expression levels between adjacent and tumor tissues were assessed by using Wilcoxon signed-rank test, as well as the adjacent and paired tumor tissues. The receiver

operating characteristic (ROC) curve was drawn to determine the diagnostic significance of AGGF1. The relationship between AGGF1 and clinical features were analyzed with Wilcoxon or Kruskal-Wallis test. The OS of high and low subgroups were compared via the Kaplan-Meier method based on log-rank tests. Univariate and multivariate Cox regression analysis were performed to verify the association between AGGF1 expression and survival along with other clinical features. *P* value less than 0.05 was considered statistically significant.



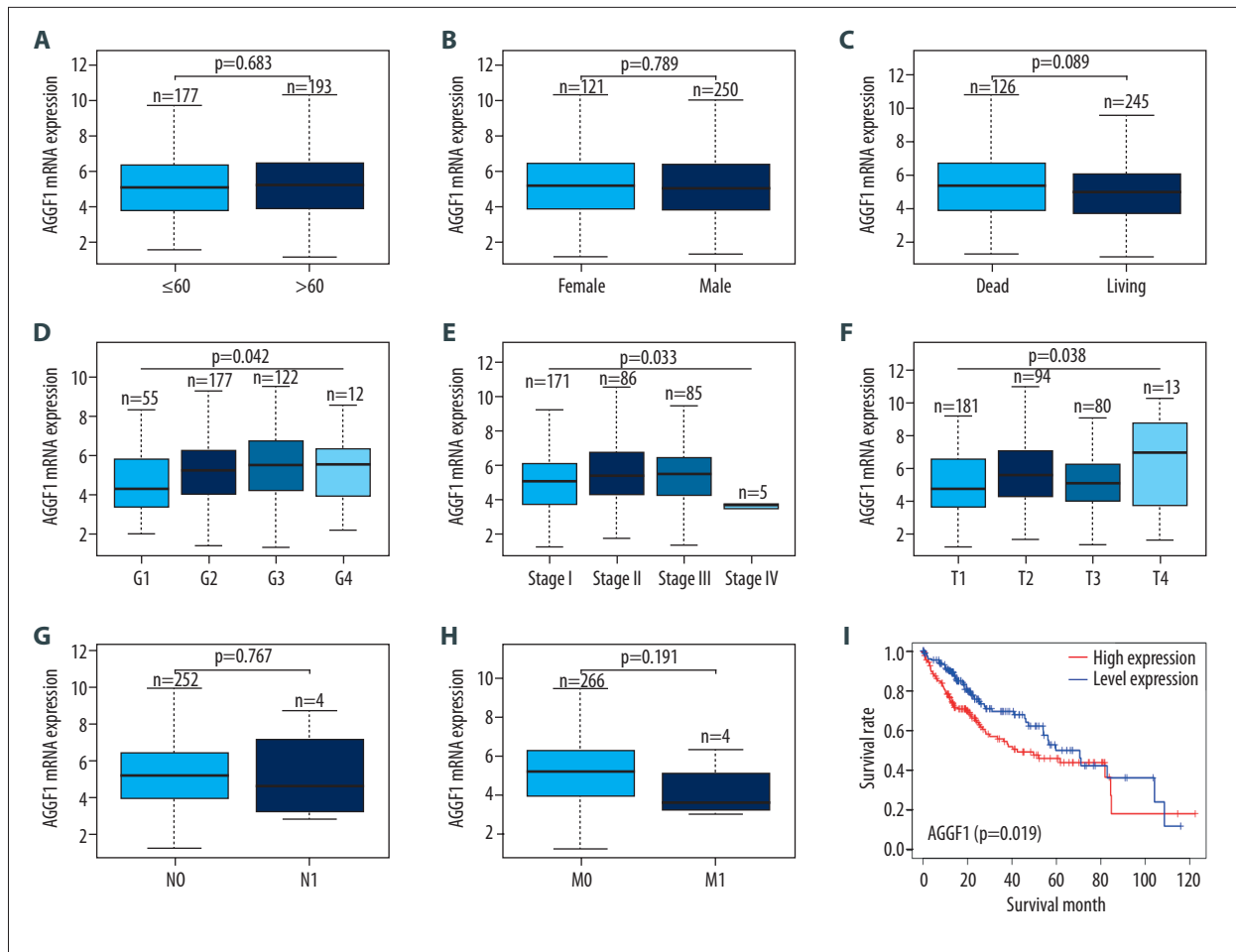
**Figure 2.** AGGF1 was upregulated in HCC and acted as a diagnostic biomarker for HCC. **(A)** AGGF1 mRNA expression in 50 adjacent normal and 374 HCC samples. **(B)** The difference between 50 HCC cases and the paired adjacent normal ones. **(C)** OncoPrint analysis of cancer versus normal tissue. The diagnostic ROC curves of AGGF1 and AFP were shown in total **(D)**, stage I & II **(E)** and stage III & IV **(F)** cohort respectively. AGGF1 – angiogenic factor with G patch and FHA domains 1; HCC – hepatocellular carcinoma; ROC – receiver operating characteristic; AFP – alpha-fetoprotein.

**Results**

**Characteristics of the study population from TCGA**

As shown in Table 1, a total of 377 HCC patients were obtained from TCGA database, including 255 male patients and 122 female patients. The average age at diagnosis was 59.5 years ( $\pm 13.5$  years) with a 34% death rate in the last

follow-up. The median time of the follow-up for patients was 19.2 months. In the TCGA cohort, the histopathologic distribution ranges from well, moderately, poorly differentiated to undifferentiated, also known as G1, G2, G3, and G4, respectively. The information of TNM stage and classification were also emerged below.



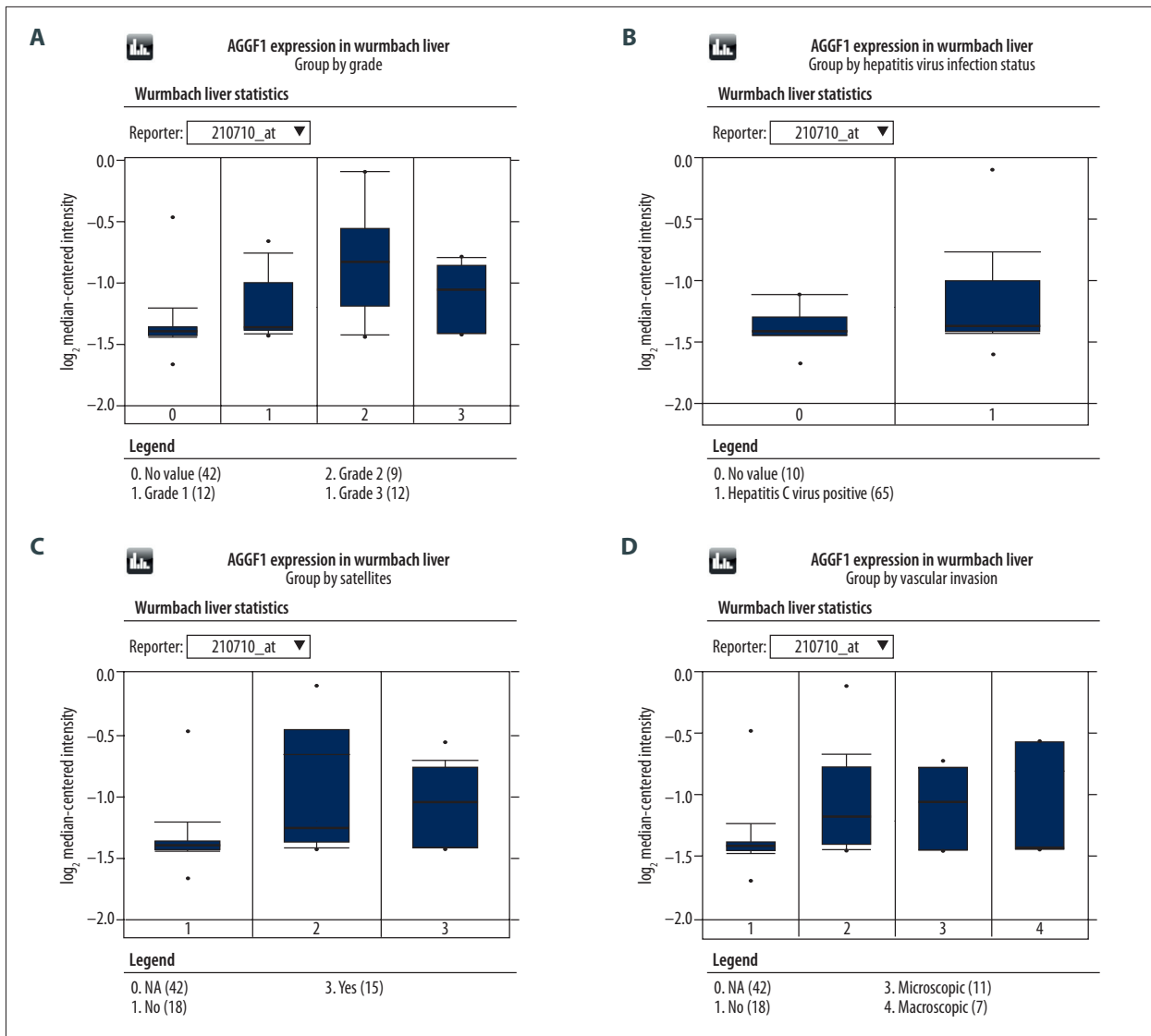
**Figure 3.** AGGF1 expression was compared in TCGA tumor cases according to patient age (A), gender (B), follow-up status (C), histological grade (D), cancer stage (E), TNM classification T (F), N (G), M (H), prognosis (I). TCGA – The Cancer Genome Atlas; AGGF1 – angiogenic factor with G patch and FHA domains 1.

### Associations between AGGF1 mRNA expression and clinical variables

Compared to normal tissues, AGGF1 mRNA expression was upregulated in multiple cancer tissues distributed in the body map (Figure 1A). In addition, we analyzed AGGF1 mRNA levels across different tumor samples paired with normal tissues (Figure 1B). A higher AGGF1 was observed in major tumors including breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), glioblastoma multiforme (GBM), liver hepatocellular carcinoma (LIHC), and pancreatic adenocarcinoma (PAAD). Surprisingly, the expression of AGGF1 is lower in liver than other normal organs (Figure 1B). Also, as shown in Figure 2A, expression levels of AGGF1 were significantly higher in 374 TCGA HCC samples compared to 50 adjacent normal samples ( $P < 0.001$ ). The results coincided well with the data obtained from comparing 50 HCC cases to corresponding adjacent tissues ( $P < 0.001$ , Figure 2B). Four different datasets (Chen liver [8], Roessler liver [9], Roessler liver 2 [9], and Wurbach

liver [10]) came to the same conclusion after comprehensive online analysis in OncoPrint ( $P = 0.002$ , Figure 2C). OncoPrint analysis of HCC versus normal tissue also showed that AGGF1 was significantly overexpressed.

Alpha-fetoprotein (AFP) is the most widely used biomarker in screening and diagnosing HCC [11]. However, we need more efficient biomarkers for HCC diagnosis. To explore the diagnostic efficacy of AGGF1, we plotted the diagnostic ROC curve of AGGF1. Meanwhile, the diagnostic efficacy of AGGF1 was compared with that of AFP. The area under the curve (AUC) of AGGF1 was 0.873 (95% confidence interval [CI]: 0.836–0.909), which is significantly larger than AFP with an AUC value 0.705 (95% CI: 0.653–0.758). We further discussed the diagnostic efficiency of AGGF1 combined with AFP. The AUC value of the combined diagnosis was 0.929 (95% CI: 0.902–0.955) ( $P < 0.05$ , Figure 2D). A more effective diagnosis was shown in the combined factors than either of AGGF1 and AFP ( $P < 0.05$ ). We finally compared the diagnostic efficacy of AGGF1 and AFP in



**Figure 4.** The association between AGGF1 expression and tumor grade (A), hepatitis virus infection status (B), satellites (C) and vascular invasion (D) in Oncomine Wurmback Liver. AGGF1 – angiogenic factor with G patch and FHA domains 1.

**Table 2.** Univariate and multivariate analysis of AGGF1 expression and clinical parameters with OS among HCC patients.

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-Value	HR	95% CI	p-Value
Age	1.01	1.00–1.03	0.177			
Gender	0.82	0.56–1.21	0.317			
Grade	1.12	0.87–1.45	0.382			
Stage	1.67	1.36–2.06	1.12E-06	1.22	0.55–2.74	0.621
T	1.65	1.36–2.01	5.82E-07	1.34	0.62–2.88	0.453
AGGF1	1.14	1.04–1.26	0.008	1.11	1.01–1.22	0.038

HR – hazard ratio; CI – confidence interval; AGGF1 – angiogenic factor with G patch and FHA domains 1; OS – overall survival; HCC – hepatocellular carcinoma.

**Table 3.** Gene sets enriched in the high AGGF1 expression phenotype.

MSigDB collection	Gene set name	ES	NES	NOM p-val	FDR q-val
c2.cp.kegg.v6.2.symbols	KEGG_REGULATION_OF_AUTOPHAGY	0.654	2.054	<0.001	0.003
	KEGG_TGF_BETA_SIGNALING_PATHWAY	0.643	2.016	<0.001	0.002
	KEGG_WNT_SIGNALING_PATHWAY	0.591	2.007	0.002	0.002
	KEGG_MTOR_SIGNALING_PATHWAY	0.644	2.005	<0.001	0.002
	KEGG_PATHWAYS_IN_CANCER	0.567	1.983	<0.001	0.003
	KEGG_CELL_CYCLE	0.684	1.979	<0.001	0.003
	KEGG_P53_SIGNALING_PATHWAY	0.584	1.908	<0.001	0.006
	KEGG_MAPK_SIGNALING_PATHWAY	0.526	1.890	<0.001	0.006
	KEGG_APOPTOSIS	0.596	1.882	<0.001	0.007

ES – enrichment score; NES – normalized ES; NOM P-val – normalized P-value; AGGF1 – angiogenic factor with G patch and FHA domains 1. Gene sets with NOM P-value <0.05 and FDR q-value <0.05 were considered as significantly enriched.

different HCC stages (Figure 2E, 2F), and the results were stable and consistent with the previous results. The high diagnostic AUC value implies that AGGF1 can effectively distinguish HCC tissue from non-tumor and the combined diagnostic model of AGGF1 and AFP was the best.

Following these initial results, we then investigated the relationship between the AGGF1 mRNA expression and the clinical characteristics of HCC patients based on TCGA cohort (Figure 3). Statistic differences in AGGF1 expression were observed according to histological grade, cancer stage, and TNM classification T ( $P<0.05$ , Figure 3D–3F). Moreover, in the Oncomine Wurmbach liver cohort, higher mRNA levels of AGGF1 were associated with worse tumor grade, positive hepatitis virus infection, satellites and vascular invasion (Figure 4).

### Prognostic valuation of AGGF1 for HCC

To evaluate the relationship between AGGF1 level and prognosis of HCC, a Kaplan-Meier survival analysis was performed in TCGA total cases (Figure 3I). The data revealed that higher mRNA expression levels of AGGF1 exhibited significantly poor OS than the lowers in HCC. The 3-year survival rate of HCC patients with high expression of ACGGF1 was 54.6% (95% CI: 46.2–64.5%), and the median survival time was 40.3 months. However, patients with low expression of AGGF1 showed 69.6% (95% CI: 61.6–78.7%) of a 3-year survival rate and 59.7 months of median survival time.

The outcomes of the univariate and multivariate Cox regression analysis to explore potential prognostic risk factors were presented in Table 2. Univariate analysis revealed that later clinical cancer stage, worse TNM classification T and high level of AGGF1 are associated with a shorter OS of HCC patient ( $P<0.05$ ).

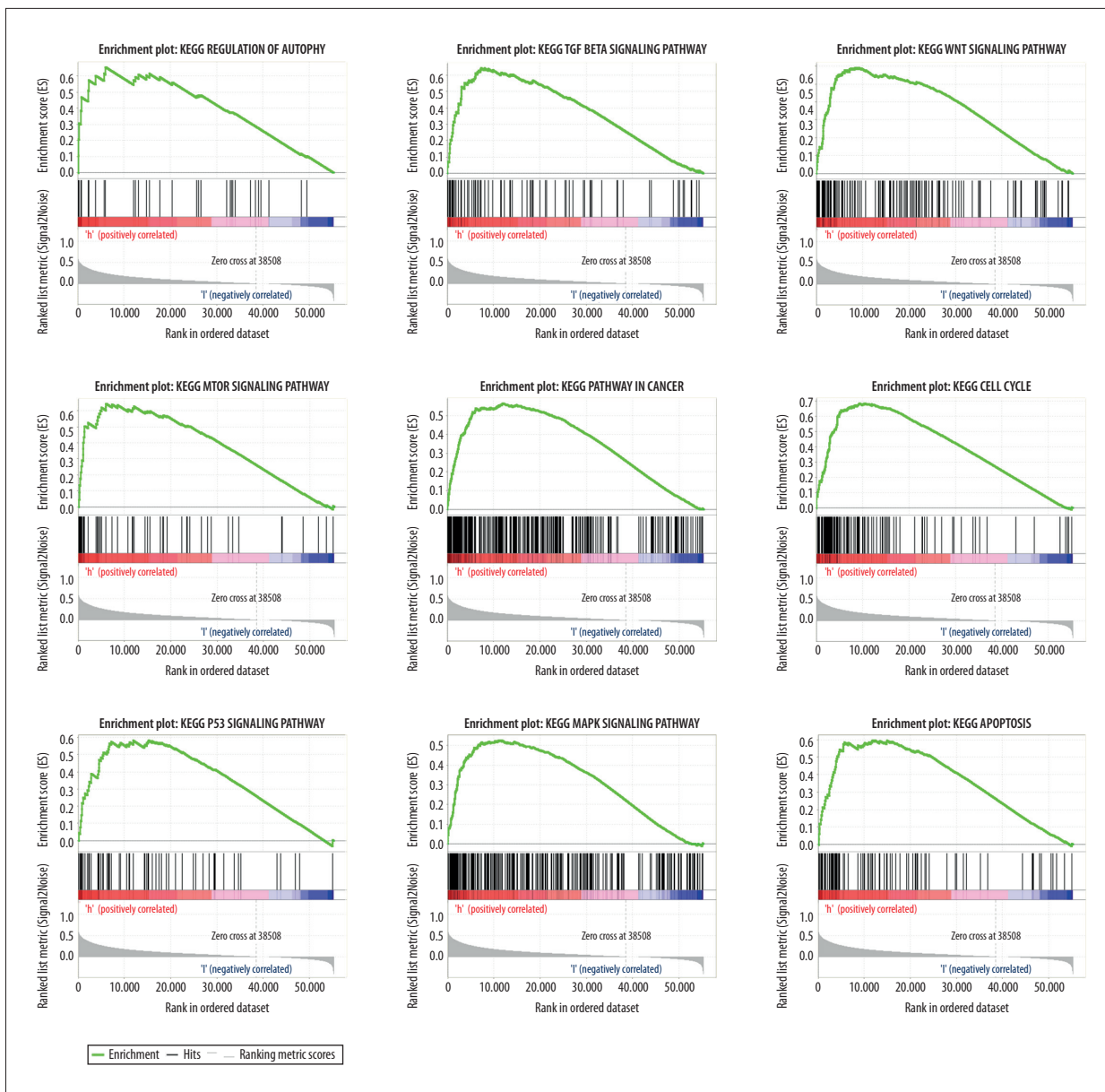
To further validate whether the 3 aforementioned factors can independently predict the prognosis of HCC, a multivariate Cox analysis was performed. The final results suggested that over-expression of AGGF1 was an independent risk factor for OS among HCC patients (HR=1.11, 95% CI: 1.01–1.22,  $P=0.038$ ).

### AGGF1-related signaling pathways identified using GSEA

As amount of dysregulated signaling pathways were responsible for tumorigenesis, a high AGGF1 mRNA level accompanied with lower survival probability may refer to a number of signaling pathways abnormally activated or inhibited by AGGF1 in HCC. We tried to elucidate the function of the AGGF1 via GSEA analysis between high and low AGGF1 expression datasets. GSEA revealed significant differences (FDR q-val <0.05, normalized  $P<0.05$ ) of many pathways observed in enrichment of MSigDB Collection (c2.cp.kegg.v6.2.symbols). The most significantly enriched signaling pathways were shown in Table 3 and Figure 5. Among these curated KEGG pathways, carcinogenesis and development associated, such as “regulation of autophagy”, “Wnt signaling pathway”, “pathway in cancer”, “cell cycle”, and “P53 signaling pathway” were differentially enriched in the high AGGF1 expression phenotype.

### Discussion

The pathogenesis of HCC is complex. The main recognized causes are chronic hepatitis virus infection, alcohol abuse injury, aflatoxin poisoning, nonalcoholic fatty liver disease, gene dysregulation, and cell dysplasia [12]. Despite accumulating achievements in treatment, the OS of HCC is still dismal [13]. In recent years, biomarkers for diagnosing and predicting patient outcome came into blossom. AFP, a well-known biomarker



**Figure 5.** Enrichment plots from the GSEA in TCGA HCC. Several pathways and biological processes were differentially enriched in high (AGGF1) expression HCC. ES – enrichment score; NES – normalized ES; NOM *P*-val – normalized *P*-value; GSEA – Gene Set Enrichment Analysis; TCGA – The Cancer Genome Atlas; HCC – hepatocellular carcinoma; AGGF1 – angiogenic factor with G patch and FHA domains 1.

for HCC is widely applied in the clinic. However, its value in diagnosis and prognosis remains controversial [14]. Therefore, seeking new efficient biomarkers for HCC diagnosis and prognosis is urgently needed.

Previous studies reported that the pro-angiogenic factor AGGF1 was a tumor promoting factor of colorectal cancer through affecting Wnt/ $\beta$ -catenin signaling pathway [15,16]. Since then, a few tentative studies were carried out to investigate the role of AGGF1 in cancer [17–20]. Immunohistochemistry was

performed in HCC and para-carcinomatous tissues in 2 separate studies [17,18]. The samples of both cohorts were small and protein levels of AGGF1 were expressed in semi-quantitative data, which could cause potential result bias. Our study firstly explored the expression and prognostic value of AGGF1 in HCC at the level of gene transcription with a large cohort.

Using online available datasets, we showed that the expression of AGGF1 in HCC was significantly different from that in normal cases. As for the phenomenon that lower expression



of AGGF1 in liver than other organs irrespective of the presence of cancer, we think it may be due to different sequencing platforms or tissue heterogeneity. AGGF1 RNA-seq profiles from TCGA and Oncomine were analyzed and revealed that high expression was positively correlated with malignant phenotype of HCC, including neoplasm histologic grade, cancer stage, satellites, vascular invasion, and survival. A negative result seen in stage N/M may be due to the single sample size of patients with lymph nodes and distant metastasis. Our result also showed a significant diagnostic value for HCC (AUC=0.873) which has not been reported previously. The diagnostic value of AGGF1 was higher than AFP (AUC=0.705,  $P<0.05$ ). Furthermore, the striking value was achieved when we used the AGGF1 and AFP together to diagnose HCC. The results were still consistent and stable according to HCC staging. We first showed that AGGF1 was a useful biomarker for HCC diagnosis better than AFP, and we showed that combining AGGF1 with AFP should be utilized to diagnose HCC in clinic in the future.

Further Kaplan-Meier survival analysis showed that higher AGGF1 expression accompanied with a significantly decreased OS. The potential mechanism may be that AGGF1 influences the signaling pathway such as “regulation of autophagy”, “Wnt signaling pathway” and “pathway in cancer” etc, as GSEA identified. Our current study implied a correlation between HCC outcome and AGGF1. As a result, it may be a new potential biomarker for predicting prognosis of HCC.

A previous study showed that AGGF1 activated autophagy in a myocardial infarction model, which played an essential role in therapeutic angiogenesis [21]. The result of GSEA also showed autophagy played a pivotal role in HCC. Several groups have reported that TGF- $\beta$  is a pro-fibrogenic stimulus. Zhou et al.

have verified that AGGF1 alleviated liver fibrosis by modulating TGF- $\beta$  signaling [22]. Together with our GSEA results, TGF- $\beta$  signaling may also be an important pathway involved in HCC with high AGGF1 expression.

Wnt/ $\beta$ -catenin signaling is a vital pathway in the development and progression of malignancies. Knockdown of AGGF1 attenuated malignant behavior of gastric cancer through Wnt/ $\beta$ -catenin pathway [23]. Another study demonstrated that expression of AGGF1 can lead to the activation of  $\beta$ -catenin-mediated transcription which is central to the formation of venous vessels [24]. We hypothesized that AGGF1 could promote tumor angiogenesis by regulating Wnt/ $\beta$ -catenin pathway. The result is consistent with our GSEA analysis of HCC tissues. The result of GSEA also showed an important role of mTOR pathway. A recent research unveiled the role of OR3A4/AGGF1/akt/mTOR axis in HCC tumor progression and angiogenesis [4].

## Conclusions

In conclusion, the present study was designed to identify whether AGGF1 was involved in the carcinogenesis or progression of HCC. AGGF1 has a high diagnostic and prognostic value for HCC together with AFP. Pathway in cancer, P53 signaling pathway, etc., may act as vital pathways regulated by AGGF1 in HCC. Nevertheless, due to the limitations in our study design, the predictive value of AGGF1 in protein levels could not be clearly assessed. Further studies are needed to elucidate the biological function of AGGF1 in HCC.

## Conflicts of interest

None.

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