Int J Clin Exp Pathol 2020;13(7):1646-1654
www.ijcep.com /ISSN:1936-2625/IJCEP0111661

Original Article

Expression of AGGF1 and Twist1 in hepatocellular carcinoma and their correlation with vasculogenic mimicry

Xu Mao[1*], Xia Wu[1*], Jiannan Guo[1*], Mingliang Deng[2], Haibo Zhang[3], Tao Ma[1], Lan Yu[1*]

[1]Department of Pathology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, Anhui, China; [2]Department of Neurosurgery, Bengbu Third People's Hospital, Bengbu, Anhui, China; [3]Dongchangfu Maternal Child Health Hospital, Liaocheng, Shandong, China. [*]Equal contributors.

Received March 30, 2020; Accepted May 20, 2020; Epub July 1, 2020; Published July 15, 2020

Abstract: Background: The most common reason for hepatocellular carcinoma (HCC) treatment failure is recurrence and metastasis. AGGF1 (a promoting gene of tumor metastasis), vasculogenic mimicry (VM, new blood supply formation in malignant tumors), and Twist1 (an evolutionarily conserved basic helix-loop-helix transcription factor) are all valuable factors for metastasis and prognosis in diverse common human cancers. However, the correlation of AGGF1, Twist1, and VM in HCC is still unclear. In this study, we analyzed the correlations among these factors as well as their correlation with clinicopathologic data and survival in HCC. Methods: Immunohistochemical (IHC) analysis was used to detect the expression of AGGF1 and Twist1 in 111 archival surgical specimens of human HCC. Furthermore, clinical data were collected. Results: Levels of VM, AGGF1 and Twist1 were significantly higher in HCC tissues than in normal hepatic tissues. Levels of VM, AGGF1, and Twist1 were positively associated with AFP, HBsAg, size, capsular invasion, Child-Pugh classification level, and tumor node metastasis (TNM) stage, and negatively associated with patients' overall survival (OS). In multivariate analysis, high levels of VM, AGGF1, Twist1, AFP, Child-Pugh classification level, as well as TNM stage were independently correlated with lower OS in patients with HCC. Conclusion: VM and the expression of AGGF1 and Twist1 may represent promising metastatic and prognostic biomarkers, as well as therapeutic targets for HCC.

Keywords: Hepatocellular carcinoma, VM, AGGF1, Twist1, prognosis

Introduction

Hepatocellular carcinoma is a common malignant tumor and the second leading cause of cancer death worldwide. High aggressiveness and metastasis, especially early intrahepatic and extrahepatic metastases, are the main reasons for poor prognosis and short survival of HCC patients. Angiogenesis is a decisive factor in the growth and spread of solid tumors such as hepatocellular carcinoma [1]. Therefore, identification of new early diagnostic tools and therapeutic methods for HCC is urgently needed [2].

Vasculogenic mimicry (VM) is a unique mode of tumor blood supply discovered by Yue [3], that is a pipeline structure that can transport blood formed by tumor cell deformation, and plays an important role in alleviating tumor hypoxia [4]. The presence of VM is associated with disease progression and poor prognosis. VM has been found in breast cancer, prostate cancer, non-small cell lung cancer, and malignant glioma. Tumors with VM structures are often associated with poor tumor progression and progression [5, 6].

Twist1 is an evolutionarily highly conserved basic helix-loop-helix (bHLH) transcription factor. Coding genes were first discovered in fruit flies in 1987. It is named Twist1 mainly because of the distortion of drosophila embryo caused by its mutation, and it regulates proenteric embryogenesis and mesenchymal development [7]. Weinberg found that Twist1 is involved in epithelial-mesenchymal transition (EMT) in 2004 and promoting tumor metastasis. Using a mouse model of breast cancer, they

Table 1. Patient characteristics

Characteristic	Frequency (n)	Percentage (%)
Age (years)		
<50	35	31.5
≥50	76	68.5
Sex		
Male	75	67.6
Female	36	32.4
Size (cm)		
<2.0	21	18.9
2.0-4.9	44	39.6
≥5.0	46	41.5
Amount		
1	91	82.0
≥2	20	18.0
Capsular invasion		
No	78	70.3
Yes	33	29.7
Child-Pugh level		
A	75	67.6
B+C	36	32.4
HBsAg		
Negative	35	31.5
Positive	76	68.5
AFP		
<400	63	56.8
≥400	48	43.2
TNM stage		
I+II	60	54.0
III+IV	51	46.0

found that increased Twist1 expression in cells [8] resulted in intercellular connections being loose, the cell movement ability was enhanced, the level of interstitial cell markers was increased, and the cells showed obvious aggressiveness [9]. Twist1 also has biologic functions such as promoting the generation of blood vessels or vascular mimicry, maintaining the characteristics of tumor stem cells, enhancing the resistance of tumor cells to chemotherapy drugs and anti-apoptotic ability [10, 11].

The AGGF1 (angiogenic factor with G and FHA domains 1) gene was discovered by Tian in 2004 with t(5; 11)(q13.3; p15.1) in 1 case. This new gene was cloned at a chromosomal heterotopic staining site during a patient's detailed molecular genetic research [12]. The gene is highly expressed in vascular endothelial cells, and the encoded protein exhibits strong angiogenic capacity in vitro. Therefore, it was preliminarily characterized as a new angiogenic factor [13]. Continuous tumor angiogenesis is one of the main signs and characteristics of liver cancer, and is closely related to its growth, invasion, metastasis, and recurrence [14, 15].

Overall, studies on VM, AGGF1, and Twist1 in relation to metastasis and prognosis have indicated that these biomarkers influence tumor development. However, associations between VM, AGGF1, and Twist1 in HCC have not yet been extensively reported. The purpose of this study was to explore the hypotheses that these biomarkers correlate with HCC metastasis and prognosis [16-18].

Methods

Patients and tissue samples

All 111 HCC tissues and corresponding matched normal tissue specimens were collected from the Department of Pathology, at the First Hospital Affiliated to Bengbu Medical College, (China) from January 2014 to December 2018 (Patients who had been administered by radio- or chemo-therapy prior to operation were excluded). The "normal" hepatic tissues were removed from the same patient, avoiding necrotic tissues, and from surrounding hepatic tissues at least 5 cm away from the tumor edge. All patients were scattered cases who had complete pathologic, clinical and follow-up data. We excluded patients who received preoperative chemotherapy or radiotherapy. All patients have complete pathology, demographics, and follow-up data (every 6 months by mobile phone and social applications). The overall survival time (OST) was calculated from surgery to death or December 2018 (mean OS: 12 months, range: 60 months). Information was obtained in writing from all patients. This study was approved by the Ethics Committee of Bengbu Medical College and conducted in accordance with the guiding principles of the Helsinki Declaration.Tumor node metastasis (TNM) was evaluated based on the eighth edition of the American Joint Committee on Cancer (AJCC). Tumor differentiation was assessed according to World Health Organization (WHO) standards. Patient characteristics are shown in **Table 1**.

Immunohistochemical analysis

Immunohistochemical staining was performed according to the instructions of EliVisionTM Plus detection kit (Lab Vision, Fremont, CA). All HCC and corresponding "normal" liver tissues were fixed in 10% buffered formalin, and then embedded in paraffin, and 4 mm thick sections were taken continuously. The samples were deparaffifinized using conventional methods and dehydrated using xylene and alcohol. Endogenous peroxidase activity was blocked with a 3% H_2O_2 in methanol solution, and the antigen was recovered with a citrate buffer. All sections were then washed several times with phosphate-buffered saline (PBS) and blocked with goat serum at room temperature for 30 min. After washing with PBS, all samples were blocked with goat serum for 20 minutes at room temperature and then incubated with mouse anti-human or AGGF1 (Abcam) for 1 hour at 37°C. All samples were blocked with goat serum for 20 minutes at room temperature, and then incubated with mouse monoclonal antibody against human AGGF1 (Abcam) or CD34 (Abcam) for 1 hour at 37°C. Periodic acid-Schiff (PAS)-CD34 double staining was used to identify endothelial cells, including vascular-like (VM) structures, in the glycosylated basement membrane of blood vessels. Finally, all sections were counterstained with hematoxylin, dehydrated, air-dried, and mounted.

Evaluation of immunostaining

All slides were evaluated by two experienced pathologists who were unaware of clinical data or disease outcomes. We performed analysis 10 representative high power fields (HPF) from each region for each HCC slide. Score experimental results were based on intensity (no staining, 0; weak staining, 1; moderate staining, and 2; strong staining, 3) and extent (<11% positive cells, 1; 11-50% positive cells, 2; 51-75% positive cells. The final score was obtained by multiplying the intensity and breadth scores, ranging from 0-12. A final score of ≥3 is considered positive. Average score of each individual in the tumor was found .

Statistical analysis

Correlations between clinicopathologic variables and AGGF1, Twist1, or VM were compared using Fisher exact test or Chi square test. The correlations among AGGF1, Twist1, or VM were compared using Spearman coefficient test. The effects of AGGF1, Twist1, or VM on survival were determined using univariate and multivariate analyses. Cox regression model was used for multivariate analysis to determine independent prognostic factors. The Kaplan-Meier method of log-rank test of univariate overall survival analysis was used. SPSS (SPSS Inc., IBM, IL) version 21.0 statistical software was used to evaluate the correlation between AGGF1 +, Twist +, VM + and clinicopathologic variables. $P<0.05$ was considered to indicate significance.

Results

Correlations between AGGF1, Twist1, VM and clinicopathologic variables

The positive expression of AGGF1 and Twist1 was found mainly on the nucleus and cytoplasm of HCC cells and corresponding normal renal tissue specimens. They were presented as a brown granular material. The positive expression of VM (42.33%, 40/111) was significantly higher than that in the control tissues (0%, 0/163; P<.05, **Figure 1A**, **1B**). The positive rate of AGGF1 expression in the HCC samples (38.70%, 43/111) was significantly higher than that in the control normal tissues (32.43%, 36/111; P<.05; **Figure 1C**, **1D**). The positive expression of Twist (40.54%, 45/111) was significantly higher than that in the control normal tissues (31.53%, 35/111; P<.05; **Figure 1E**, **1F**). The positive expression rate of AGGF1, Twist1 and VM in HCC was positively correlated with size, capsular invasion, Child-Pugh classification level of tumors (P<.05) and AFP, HBsAg, and TNM stage (P<.05), but not with patient age, gender, or amount of tumors (**Table 2**).

Univariate and multivariate analysis

Follow-up data suggested that OST was significantly shorter in HCC patients with VM positive specimens (26.93±3.26 months) than in those with VM negative specimens (38.65± 2.72 months; log rank = 9.233, P<.05; **Figure 2A**). The OS time of Twist1 positive patients (23.71±2.95 months) was significantly shorter than those who were Twist1 negative (41.87± 2.68 months; log rank = 22.079, P<.05; **Figure 2B**). Similarly, the OS time of AGGF1 positive patients (25.33±3.17 months) was significant-

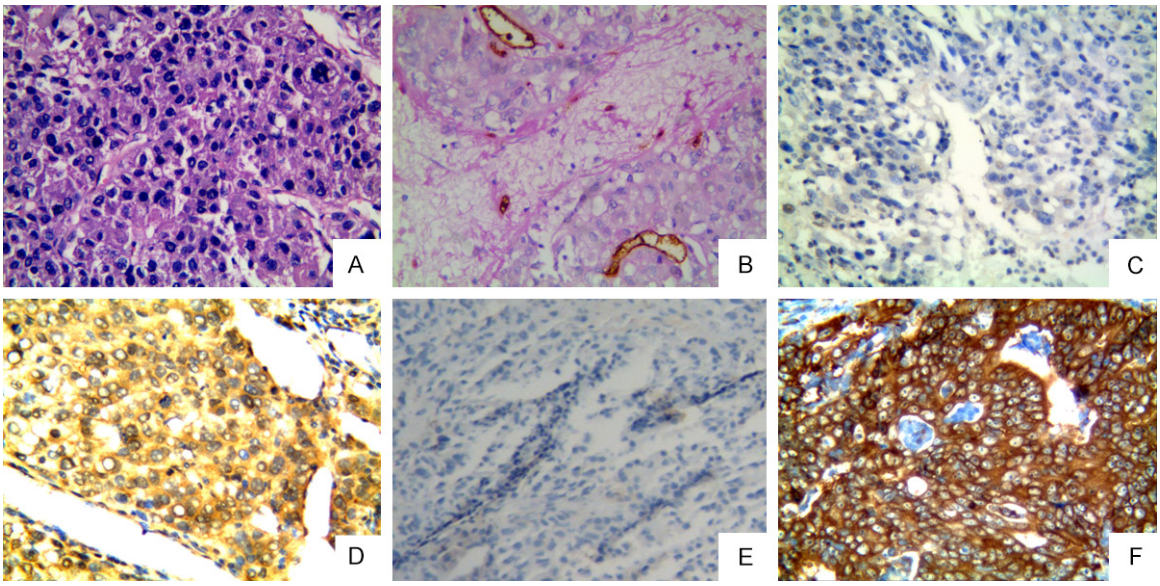

Figure 1. Immunostaining of AGGF1, or Twist1 or VM in HCC or the control tissue. A. Negative staining of VM in HCC (400× magnification); B. Positive staining of VM in the control tissue (400× magnification); C. Negative staining of AGGF1 in HCC (400× magnification); D. Positive staining of AGGF1 in the control tissue (400× magnification); E. Negative staining of Twist1 in the HCC (400× magnification); F. Positive staining of Twist1 in the control tissue (400× magnification).

ly lower than those of AGGF1 negative patients (40.00±2.69 months; log-rank = 15.054, P< .05; **Figure 2C**). The combination of AGGF1 and Twist1 and VM positive expression had a poorer prognosis than did the reverse combination (log-rank = 15.054, P<.05; **Figure 2G**). In the univariate analysis, OS time was significantly correlated with clinicopathologic variables, including Child-Pugh classification level (P<.05, log-rank = 5.796; **Figure 2D**), TNM (P<.05, log-rank = 5.900; **Figure 2E**), AFP (P<.05, log-rank = 4.883; **Figure 2F**) (**Table 3**). Multivariate analysis suggested that AGGF1, Twist1, and VM, AFP, TNM Stage and Child-Pugh classification level as well as TNM were independent prognostic indicators for HCC (**Table 4**).

Correlation among the expression of AGGF1, TWIST1, and VM in HCC

Association among AGGF1, TWIST1, and VM in HCC Spearman correlation coefficient analysis indicated a positive association between the positive expression of VM and that of AGGF1 (r=0.360, P<.05), Twist1 (r=0.336, P<.05), or VM (r=0.328, P<.05) (**Table 5**).

Discussion

The AGGF1 gene is the first gene discovered and cloned during genetic linkage analysis of Klippel-Trenaunay syndrome (KTS) [19]. The AGGF1 gene encodes a new angiogenic factor protein, which contains an FHA domain and a G-PATCH domain to promote angiogenesis. AGGF1 protein is highly expressed in endothelial cells, smooth muscle cells, and osteoblasts, and higher expression levels of AGGF1 can be detected in blood vessels of various tissues [20]. Continuous angiogenesis is one of the main characteristics of primary liver cancer, and studies have shown that AGGF1 can promote angiogenesis and improve ischemic function, which provides conditions for the further growth, invasion, and metastasis of tumors. We propose that AGGF1 plays a role in promoting the growth, metastasis and invasion of liver cancer. In our study, AGGF1 accounted for 38.74% of the 111 cases, and was closely related to the growth of tumor tissues [21]. The more poor the tumor differentiation, the larger the tumor volume, and the more obvious [22]. In the later clinical stages, the expression level was also increased. The difference was statistically significant. Survival analysis also showed that the survival time of HCC patients with AGGF1 positive expression was significantly shorter than that of those who were negative, which was consistent with the relevant literature. The abnormal expression of AGGF1 gene is closely related to occurrence and develop-

Table 2. Association between VM and expression of AGGF1, Twist1, and clinicopathologic characteristics of HCC

Variable	AGGF1		P	TWIST1		P	VM		P
	Negative	Positive		Negative	Positive		Negative	Negative	
Sex									
Female	24	12	0.418	19	17	0.321	22	14	0.664
Male	44	31		47	28		49	26	
Age, y									
<50	21	14	0.853	20	15	0.736	21	14	0.555
≥50	47	29		46	30		50	26	
Size									
<2.0	18	3	0.012	19	2	0.004	18	3	0.028
2.0-4.9	28	16		25	19		29	15	
≥5.0	22	24		22	24		24	22	
Amount									
1	54	37	0.376	55	36	0.654	61	30	0.151
≥2	14	6		11	9		10	10	
Capsular invasion									
No	61	17	0.000	55	23	0.000	58	20	0.000
Yes	7	26		11	22		13	20	
Child level									
A	51	24	0.035	51	24	0.008	55	20	0.003
B+C	17	19		15	21		16	20	
AFP									
<400	45	18	0.012	45	18	0.003	49	14	0.001
≥400	23	25		21	27		22	26	
HBsAg									
Negative	29	6	0.002	26	9	0.031	29	6	0.005
Positive	39	37		40	36		42	34	
TNM Stage									
I+II	44	16	0.005	43	17	0.004	44	16	0.026
III+IV	24	27		23	28		27	24	

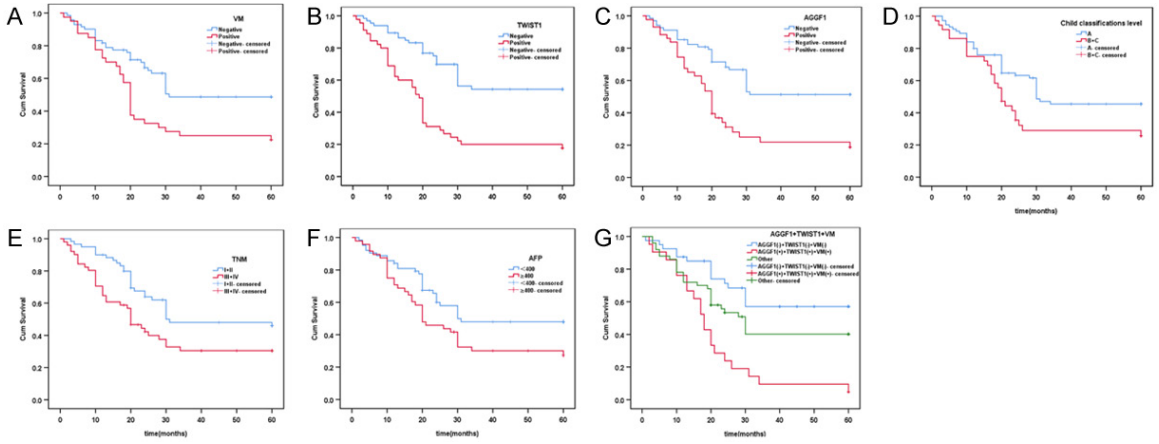

Figure 2. Kaplan-Meier analysis of the survival rate of patients with HCC. (A) Overall survival of all patients in relation to VM expression (log-rank =9.233, P<.05); (B) Overall survival of all patients in relation to Twist1 (log-rank =22.079,

P<.05); (C) Overall survival of all patients in relation to AGGF1 (log-rank =15.054, P<.05); (D) Overall survival of all patients in relation to Child level (log-rank =5.796, P<.05. The blue line represents patients with (A), the red line represents patients with (B+C)). (E) Overall survival of all patients in relation to TNM stage (log-rank =5.900, P<.05. The blue line represents patient with I+II stage group, the red line represents patients with III+IV stage group). (F) Overall survival of all patients in relation to AFP (log-rank =4.883, P<.05. The blue line represents patient with AFP less than 400 ng/ml, the red line represents patient with AFP more than 400 ng/ml). (G) Overall survival of all patients in relation to the combination of VM, Twist1 and AGGF1 (log-rank =15.054, P<.05). The red line represents positive expression of VM, Twist1 and AGGF1 and the blue line represents negative expression of VM, Twist1 and AGGF1. The green line represents other positive or negative expression of the proteins.

Table 3. Results of univariate analyses of overall survival (OS) time

Variable	n	Mean, OS	Log-rank	P
Sex				
Female	36	38.34±3.86	1.391	0.238
Male	75	32.50±2.59		
Age				
<50	35	30.23±3.70	1.491	0.222
≥50	76	36.26±2.65		
Size				
<2.0	21	46.32±4.78	7.928	0.019
2.0-4.9	44	33.71±3.46		
≥5.0	46	29.64±3.10		
Amount				
1	91	34.85±2.35	0.488	0.485
≥2	20	31.87±5.55		
Capsular invasion				
No	78	38.41±2.52	12.060	0.001
Yes	33	24.93±3.68		
Child level				
A	75	37.32±2.61	5.796	0.016
B+C	36	28.30±3.67		
AFP				
<400	63	38.09±2.90	4.883	0.027
≥400	48	29.60±3.14		
HBsAg				
Negative	35	40.50±3.64	4.067	0.044
Positive	76	31.44±2.63		
TNM Stage				
I+II	60	39.24±2.83	5.900	0.015
III+IV	51	28.57±3.17		
AGGF1				
Negative	68	40.00±2.69	15.054	0.000
Positive	43	25.33±3.17		
TWIST1				
Negative	66	41.87±2.68	22.079	0.000
Positive	45	23.71±2.95		
VM				
Negative	71	38.65±2.72	9.233	0.002
Positive	40	26.93±3.26		

ment of HCC. We conclude that the tumors expressing AGGF1 protein are poorly differentiated and have advanced clinical stage [23].

In the process of tumor growth, invasion, and metastasis, sufficient oxygen and nutrients are required [24-26]. The classic theory of tumor angiogenesis is that tumors can form blood vessels either through the budding of original vascular endothelial cells, or by inducing vascular endothelial precursor cells to differentiate into endothelial cells [27-29]. In 1999, when Maniotis et al. studied uveal melanoma, they found a duct-like shape that is not surrounded by vascular endothelial cells, but is surrounded by plastic tumor cells that mimic vascular endothelial cells and extracellular matrix remodeling [30]. Through this pipeline-like structure oxygen and nutrition are obtained;this is VM [31-33]. In this study, 40 patients with HCC met the VM standard structure, confirming the presence of VM in HCC. It is suggested that VM is closely related to the existence of tumor, as well as the degree of tumor differentiation and tumor size. Survival analysis also shows that the time of VM negative expression in HCC patients was significantly longer than that in the positive expression group. We can conclude that VM tumors are poorly differentiated.

In recent years, a large number of studies have shown that Twist1 is involved in the development of many cancers [34, 35]. For example, TWIST1 can inhibit cell senescence and apoptosis through multiple pathways, induce EMT, promote tumor invasion and metastasis, and promote angiogenesis or angiogenesis mimicry (vasculogenic mimicry, VM), maintaining the characteristics of tumor stem cells, and enhancing resistance to tumor chemotherapy drugs [36]. Twist1 also has biologic functions such as promoting the generation of blood vessels or vascular mimicry, maintaining the characteristics of tumor stem cells, and enhancing

Table 4. Results of multivariate analyses of overall survival (OS) time

Variable	B	SE	Wald	P	RR	95% CI
Child level	0.534	0.257	4.295	0.038	1.705	1.029-2.824
TNM Stage	0.517	0.253	4.174	0.041	1.676	1.021-2.752
AFP	0.532	0.251	4.495	0.034	1.702	1.041-2.783
AGGF1	0.658	0.273	5.802	0.016	1.932	1.131-3.301
TWIST1	0.901	0.271	11.093	0.001	2.462	1.449-4.184
VM	0.726	0.251	8.344	0.004	2.066	1.263-3.381

Table 5. Correlation among VM, expression of AGGF1 and Twist1 in HCC

	AGGF1			TWIST1			VM	
	Negative	Positive		Negative	Positive		Negative	Positive
TWIST1			VM			AGGF1		
Negative	50	16	Negative	51	20	Negative	52	16
Positive	18	27	Positive	15	25	Positive	19	24
r	0.360		r	0.336		r	0.328	
P	0.000		P	0.000		P	0.000	

the resistance of tumor cells to chemotherapy drugs and anti-apoptotic ability [37-39]. Numerous experimental studies have confirmed this, and activation of EMT can promote cell invasion and migration of tumor cells. In addition, many molecules have been shown to participate in EMT and in certain signal transduction pathways through this process [40]. EMT can promote the formation of VM through different signaling pathways, and related transcription factors include Twist1, ZEB1, Snail, and Slug [41]. EMT can also induce the formation of VM in tumors. Cells have a synergistic effect under hypoxic conditions, which is closely related to tumor aggressive biologic behavior and increases the mortality of tumor patients [42].

In this study, we analyzed the correlation between the expression of AGGF1 protein and Twist1 and VM, and found that with the elevation of KAI1-positive rate in tumor tissue, the positive rate of Twist1 also increased. At the same time, the positive rate of VM increased. It is suggested that the expression of AGGF1 may be related to the positive rate of Twist1 and VM. With tumor progression, tumor tissue is prone to hypoxia and ischemia, and this process will induce the formation of VM. AGGF1 expression increase at this time will result in cell adhesion weakening and poor cell differentiation. The channel and VM structure with tumor cells is only separated by a layer of PAS-positive substances. Tumor cells with low adhesion are easily detached under the influence of blood flow, leading to lymph node metastasis and distant metastasis. However, the number of specimens in our study was relatively small. Further study of large samples, associated cytological experiments and molecular experiments are necessary to validate current observations.

Conclusions

We examined the roles of VM, AGGF1, and Twist1 in HCC. High AGGF1 expression combined with high VM and Twist1 was associated with metastasis and a poor prognosis in HCC. Furthermore, VM, AGGF1 and Twist1 might serve as valuable biomarkers in HCC, and the comprehensive detection of VM, AGGF1, and Twist1 may indicate prognosis.

Acknowledgements

We thank all colleagues in the Department of Pathology, the First Hospital Affiliated to Bengbu Medical College for their help and support in this study. This study was supported by Bengbu Medical College innovation plan natural science project (Byycx1971). All authors have contributed greatly, and all authors are in agreement with the content of the manuscript.

Disclosure of conflict of interest

None.

Abbreviations

VM, vasculogenic mimicry; HCC, hepatocellular carcinoma; OS, overall survival; TNM, tumor node metastasis.

Address correspondence to: Lan Yu, Departments of Pathology, The First Affiliated Hospital of Bengbu Medical College, 287 Changhuai Road, Anhui, China. Tel: +86-18555520163; E-mail: yulan790210@163.com

References

[1] Li X, Sun B, Zhao X, An J, Zhang Y, Gu Q, Zhao N, Wang Y and Liu F. Function of BMP4 in the formation of vasculogenic mimicry in hepatocellular carcinoma. J Cancer 2020; 11: 2560-2571.

[2] Forner A, Llovet JM and Bruix J. Hepatocellular carcinoma. Lancet 2012; 379: 1245-1255.

[3] Yue WY and Chen ZP. Does vasculogenic mimicry exist in astrocytoma? J Histochem Cytochem 2005; 53: 997-1002.

[4] Liu WB, Xu GL, Jia WD, Li JS, Ma JL, Chen K, Wang ZH, Ge YS, Ren WH, Yu JH, Wang W and Wang XJ. Prognostic significance and mechanisms of patterned matrix vasculogenic mimicry in hepatocellular carcinoma. Med Oncol 2011; 28 Suppl 1: S228-38.

[5] Racordon D, Valdivia A, Mingo G, Erices R, Aravena R, Santoro F, Bravo ML, Ramirez C, Gonzalez P, Sandoval A, González A, Retamal C, Kogan MJ, Kato S, Cuello MA, Osorio G, Nualart F, Alvares P, Gago-Arias A, Fabri D, Espinoza I, Sanchez B, Corvalán AH, Pinto MP and Owen GI. Structural and unctional identification of vasculogenic mimicry in vitro. Sci Rep 2017; 7: 6985.

[6] Cao Z, Bao M, Miele L, Sarkar FH, Wang Z and Zhou Q. Tumour vasculogenic mimicry is associated with poor prognosis of human cancer patients: a systemic review and meta-analysis. Eur J Cancer 2013; 49: 3914-3923.

[7] Thisse B, el Messal M and Perrin-Schmitt F. The twist gene: isolation of a drosophila zygotic gene necessary for the establishment of dorsoventral pattern. Nucleic Acids Res 1987; 15: 3439-3453.

[8] Deng JJ, Zhang W, Xu XM, Zhang F, Tao WP, Ye JJ and Ge W. Twist mediates an aggressive phenotype in human colorectal cancer cells. Int J Oncol 2016; 48: 1117-1124.

[9] Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A and Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 2004; 117: 927-939.

[10] Lee TK, Poon RT, Yuen AP, Ling MT, Kwok WK, Wang XH, Wong YC, Guan XY, Man K, Chau KL and Fan ST. Twist overexpression correlates with hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. Clin Cancer Res 2006; 12: 5369-5376.

[11] Yuen HF, Chua CW, Chan YP, Wong YC, Wang X and Chan KW. Significance of TWIST and E-cadherin expression in the metastatic progression of prostatic cancer. Histopathology 2007; 50: 648-658.

[12] Tian XL, Kadaba R, You SA, Liu M, Timur AA, Yang L, Chen Q, Szafranski P, Rao S, Wu L, Housman DE, DiCorleto PE, Driscoll DJ, Borrow J and Wang Q. Identification of an angiogenic factor that when mutated causes susceptibility to Klippel-Trenaunay syndrome. Nature 2004; 427: 640-645.

[13] Chen D, Li L, Tu X, Yin Z and Wang Q. Functional characterization of Klippel-Trenaunay syndrome gene AGGF1 identifies a novel angiogenic signaling pathway for specification of vein differentiation and angiogenesis during embryogenesis. Hum Mol Genetics 2013; 22: 963-976.

[14] Lu Q, Yao Y, Yao Y, Liu S, Huang Y, Lu S, Bai Y, Zhou B, Xu Y, Li L, Wang N, Wang L, Zhang J, Cheng X, Qin G, Ma W, Xu C, Tu X and Wang Q. Angiogenic factor AGGF1 promotes therapeutic angiogenesis in a mouse limb ischemia model. PLoS One 2012; 7: e46998.

[15] Li L, Chen D, Li J, Wang X, Wang N, Xu C and Wang QK. Aggf1 acts at the top of the genetic regulatory hierarchy in specification of hemangioblasts in zebrafish. Blood 2014; 123: 501-508.

[16] Paulis YW, Soetekouw PM, Verheul HM, Tjan-Heijnen VC and Griffioen AW. Signalling pathways in vasculogenic mimicry. Biochim Biophys Acta 2010; 1806: 18-28.

[17] Sun T, Zhao N, Zhao XL, Gu Q, Zhang SW, Che N, Wang XH, Du J, Liu YX and Sun BC. Expression and functional significance of Twist1 in hepatocellular carcinoma: its role in vasculogenic mimicry. Hepatology 2010; 51: 545-556.

[18] Lu Q, Yao Y, Yao Y, Liu S, Huang Y, Lu S, Bai Y, Zhou B, Xu Y, Li L, Wang N, Wang L, Zhang J, Cheng X, Qin G, Ma W, Xu C, Tu X and Wang Q. Angiogenic factor AGGF1 promotes therapeutic angiogenesis in a mouse limb ischemia model. PLoS One 2012; 7: e46998.

[19] Yao HH, Wang BJ, Wu Y and Huang Q. High expression of angiogenic factor with G-patch and FHA domain1 (AGGF1) predicts poor prognosis

in gastric cancer. Med Sci Monit 2017; 23: 1286-94.
[20] Wang W, Li GY, Zhu JY, Huang DB, Zhou HC, Zhong W and Ji CS. Overexpression of AGGF1 is correlated with angiogenesis and poor prognosis of hepatocellular carcinoma. Med Oncol 2015; 32: 131.
[21] Xu Y, Zhou M, Wang J, Zhao Y, Li S, Zhou B, Su Z, Xu C, Xia Y, Qian H, Tu X, Xiao W, Chen X, Chen Q and Wang QK. Role of microrna-27a in down-regulation of angiogenic factor aggf1 under hypoxia associated with high-grade bladder urothelial carcinoma. Biochim Biophys Acta 2014; 1842: 712-725.
[22] Zhang T, Yao Y, Wang J, Li Y, He P, Pasupuleti V, Hu Z, Jia X, Song Q, Tian XL, Hu C, Chen Q and Wang QK. Haploinsufficiency of Klippel-Trenaunay syndrome gene Aggf1 inhibits developmental and pathological angiogenesis by inactivating PI3K and AKT and disrupts vascular integrity by activating VE-cadherin. Hum Mol Genet 2016; 25: 5094-110.
[23] Tu J, Ying X, Zhang D, Weng Q, Mao W, Chen L, Wu X, Tu C, Ji J and Huang Y. High expression of angiogenic factor AGGF1 is an independent prognostic factor for hepatocellular carcinoma. Oncotarget 2017; 8: 111623-111630.
[24] Yao XH, Ping YF and Bian XW. Contribution of cancer stem cells to tumor vasculogenic mimicry. Protein Cell 2011; 2: 266-72.
[25] Yue WY and Chen ZP. Does vasculogenic mimicry exist in astrocytoma? J Histochem Cytochem 2005; 53: 997-1002.
[26] Yilmaz M and Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009; 28: 15-33.
[27] Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, Baba H and Mori M. Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer Sci 2010; 101: 293-9.
[28] Lamouille S, Xu J and Derynck R. Molecular mechanisms of epithelial mesenchymal transition. Nat Rev Mol Cell Biol 2014; 15: 178-196.
[29] Ma JL, Han SX, Zhu Q, Zhao J, Zhang D, Wang L and Lv Y. Role of wist in vasculogenic mimicry formation in hypoxic hepatocellular carcinoma cells in vitro. Biochem Biophys Res Commun 2011; 408: 0-691.
[30] Liu Q, Qiao L, Liang N, Xie J, Zhang J, Deng G, Luo H and Zhang J. The relationship between vasculogenic mimicry and epithelial-mesenchymal transitions. J Cell Mol Med 2016; 20: 1761-1769.
[31] Zhang S, Zhang D and Sun B. Vasculogenic mimicry: current status and future prospects. Cancer Lett 2007; 254: 157-64.
[32] Paulis YW, Soetekouw PM, Verheul HM, Tjan-Heijnen VC and Griffioen AW. Signalling pathways in vasculogenic mimicry. Biochim Biophys Acta 2010; 1806: 18-28.
[33] Qiao L, Liang N, Zhang J, Xie J, Liu F, Xu D, Yu X and Tian Y. Advanced research on vasculogenic mimicry in cancer. J Cell Mol Med 2015; 19: 315-26.
[34] Khan MA, Chen HC, Zhang D and Fu J. Twist: a molecular target in cancer therapeutics. Tumour Biol 2013; 34: 2497-2506.
[35] Zhu QQ, Ma C, Wang Q, Song Y and Lv T. The role of TWIST1 in epithelial-mesenchymal transition and cancers. Tumour Biol 2016; 37: 185-197.
[36] Cheng GZ, Zhang W and Wang LH. Regulation of cancer cell survival, migration, and invasion by Twist: AKT2 comes to interplay. Cancer Res 2008; 68: 957-960.
[37] Eckert MA, Lwin TM, Chang AT, Kim J, Danis E, Ohno-Machado L and Yang J. Twist1-induced invadopodia formation promotes tumor metastasis. Cancer Cell 2011; 19: 372-386.
[38] Sun B, Zhang D, Zhao N and Zhao X. Epithelial-to-endothelial transition and cancer stem cells: two cornerstones of vasculogenic mimicry in malignant tumors. Oncotarget 2017; 8: 30502-30510.
[39] Zhang Z, Xie D, Li X, Wong YC, Xin D, Guan XY, Chua CW, Leung SC, Na Y and Wang X. Significance of TWIST expression and its association with E-cadherin in bladder cancer. Hum Pathol 2007; 38: 598-606.
[40] Mikheeva SA, Mikheev AM, Petit A, Beyer R, Oxford RG, Khorasani L,Maxwell JP, Glackin CA, Wakimoto H, Gonzalez-Herrero I, Sanchez-Garcia I, Silber JR, Horner PJ and Rostomily RC. TWIST1 promotes invasion through mesenchymal change in human glioblastoma. Mol Cancer 2010; 9: 194.
[41] Jung HY and Yang J. Unraveling the TWIST between EMT and cancer stemness. Cell Stem Cell 2015; 16: 1-2.
[42] Tseng JC, Chen HF and Wu KJ. A twist tale of cancer metastasis and tumor angiogenesis. Histol Histopathol 2015; 30: 1283-94.