

Correlation of Preoperative Gd-EOB-DTPA-Contrast-Enhanced MRI With TGF- β 1 Expression in Hepatocellular Carcinoma

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Research Article

Keywords: Hepatocellular carcinoma, magnetic resonance imaging (MRI), Gd-EOB-DTPA-enhanced MRI, TGF- β 1, targeted cancer therapy

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Correlation of preoperative Gd-EOB-DTPA-contrast-enhanced MRI with TGF- β 1 expression in hepatocellular carcinoma

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Abstract

Purpose: To investigate the feasibility of preoperative gadolinium ethoxy-benzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) -enhanced magnetic resonance imaging (MRI) related signs in predicting transforming growth factor- β 1(TGF- β 1) gene expression in hepatocellular carcinoma (HCC).

Materials and methods: Sixty patients with HCC (55 males, mean age 52.6 ± 12 years) who underwent preoperative MRI enhancement were retrospectively analyzed. Qualitative and quantitative features of Gd-EOB-DTPA-enhanced MRI in these pathologically confirmed HCC patients were analyzed. Reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) were performed to determine the mRNA and protein levels of TGF- β 1 in HCC. The relationship between these imaging features and TGF- β 1 expression level in HCC was evaluated by rank sum test, correlation analysis and logistic regression analysis.

Results: RT-PCR indicated that the expression level of TGF- β 1 mRNA was statistically significant with the change of hepatobiliary signal ($P=0.009$). Immunohistochemical results indicated that the apparent diffusion coefficient (ADC) value of tumor was statistically significant at the protein level ($P=0.038$). There was no statistical significance in the distribution of other image features. Binary logistic regression analysis suggested that heterogeneous signals in hepatobiliary phase were independent risk factors for high TGF- β 1 expression in HCC ($P=0.012$, OR=5.333, 95%CI:1.437-19.801). Multivariate logistic regression analysis showed that ADC value was not an independent risk factor for TGF- β 1 expression.

Conclusions: heterogeneous signal performance in the hepatobiliary phase and/or ADC values of HCC are potential indicators of high

expression of TGF- β 1. Gd-EOB-DTPA-enhanced MRI may be useful in the selection of targeted therapies for patients with HCC.

Keywords: Hepatocellular carcinoma, magnetic resonance imaging (MRI), Gd-EOB-DTPA-enhanced MRI, TGF- β 1, targeted cancer therapy

Introduction

HCC is one of the leading reasons of cancer-related deaths worldwide[1]. For advanced HCC, targeted therapy is the main treatment or adjuvant method commonly used in clinical practice. However, due to the existence of tumor genetic heterogeneity, a large proportion of patients cannot benefit from targeted therapy. Most patients do not have a good prognosis due to recurrence or metastasis after surgery. Therefore, it is very important to identify markers or target gene expression that can predict the efficacy of targeted therapy. TGF- β 1 is a multifunctional cytokine that regulates important cellular processes such as angiogenesis, proliferation and differentiation, as well as immunosuppression, tissue development, and extracellular matrix formation. TGF- β 1 can promote the epithelial-to-mesenchymal transformation(EMT) of hepatocytes, leading to the progression of liver fibrosis [2].In addition, TGF- β 1 has a dual role, inhibiting the occurrence of liver tumors by inducing cell proliferation and apoptosis in the early stage. However, TGF- β 1 can promote the growth of late stage tumors and promote the invasion, metastasis and drug resistance of cancer cells by promoting epithelial-mesenchymal transformation, leading to malignant progression [3].TGF- β 1 is an independent prognostic factor for hepatocellular carcinoma, and its up-regulated expression in cancer is associated with poor prognosis[4, 5], and may be a potential target for adjuvant therapy[6].Currently, several inhibitors of the TGF- β signaling pathway, such as receptor kinase inhibitors, ligand traps, and neutralizing antibodies, have been undergoing clinical trials [3, 7]. A

phase 2 clinical trial of TGF- β showed elevated TGF- β levels in 35% of hepatocellular carcinomas, and patients with high levels of TGF- β and alpha-fetoprotein significantly improved overall survival by up to 21 months compared to the current 9-11 months survival rate in patients with advanced HCC[8, 9]. Other studies have shown that the combination of PD-1/PD-L1 with TGF- β inhibitor can improve the activity of tumor immunotherapy [10].

At present, preoperative genetic testing in HCC patients is mainly performed by biopsy. If TGF- β expression can be predicted by non-invasive methods, such as enhanced MRI related signs, it will be of great significance for the decision-making of drug therapy in clinical HCC patients. The aim of this study was to assess which enhanced MR image features were associated with TGF- β 1 expression at the transcriptional and protein levels.

Materials and methods

Study patients

We retrospectively collected and analyzed 60 patients with HCC who were diagnosed and treated in the First Affiliated Hospital of Guangxi Medical University (Guangxi, China) spanning from April 2019 to December 2020. Inclusion criteria were as follows : (1) HCC was pathologically diagnosed and confirmed after surgery; (2) Pre-operative Gd-EOB-DTPA enhanced MRI examination was performed; (3) No distant metastasis; (4) Surgical treatment was conducted within 2 weeks after the enhanced MRI examination; (5) HCC was not treated before MR examination and surgical resection. The patients with the following conditions were excluded in this study: (1) No pre-operative Gd-EOB-DTPA enhanced MRI scan; (2) medical history with other malignant tumors; (3) incomplete pathological and/or clinical information; (4) poor quality of MRI images.

MRI acquisition and image analysis

All patients underwent enhanced MRI examination on the 3.0 T MR scanner (German Siemens) 2 weeks before surgery, the contrast medium was Gd-EOB-DTPA (Bayer Healthcare Co., Ltd., Germany) .Gd-

EOB-DTPA (dosage, 0.025 mmol/kg) was injected at a flow rate of 2 ml/s through the anterior cubital vein. Normal saline (20 ml) was then injected at the flow rate of 2 ml/s for flushing. The following main sequences were used: T1-weighted image (T1WI), T2-weighted image (T2WI), ADC image, diffusion-weighted image (DWI, $b = 50, 800 \text{ s/mm}^2$), T1 mapping. The arterial phase, portal vein phase, equilibrium phase, and hepatobiliary phase were scanned 14-20 seconds, 40-50 seconds, 90-120 seconds, and 20 minutes after contrast agent injection, respectively. Two experienced radiologists analyzed MRI images blind to pathological findings. Qualitative MRI features, including tumor thrombosis, T2WI and hepatobiliary phase signal intensity, tumor margin, tumor capsule status, Intratumoral vessels, Intratumoral hemorrhage and intratumoral fat were determined by two radiologists. Qualitative MRI features were characterized (Fig. 1):(1) Tumor thrombosis: portal vein or hepatic vein filling defect, obvious enhancement at the phase of arterial, obvious flushing at the phase of portal vein;(2) Signal intensity: T2WI transverse signal intensity uniformity was evaluated, and hepatobiliary phase signals were evaluated by hypointensity, heterogeneous of high and low signal; (3) tumor capsule: The high signal thin margin of the tumor margin in the equilibrium phase is considered as the tumor capsule. Tumors are defined as either capsule or not capsule according to the capsule sign. The capsule neoplasms were further divided into completely capsule and incomplete capsule groups; (4) tumor margin: Depending on the edge of the hepatobiliary phase, there are four types of tumor margin : smooth isolated type, protruding type, irregular infiltrating and multiple nodular fusion type; (5) intratumoral vessels: Blood vessels can be seen within the tumor in the arterial phase. Quantitative characteristics included tumor size in diameter, ADC value(according to DWI with $b=800 \text{ s/mm}^2$), T1 percentage decline (T1D%). Based on coronal and axial images, the maximum tumor diameter was measured at the phase of hepatobiliary. ADC values, non-enhanced T1 relaxation time (T1N) and hepatobiliary phases (T1E) were all at the solid component level of HCC to avoid necrosis, blood vessels

and artifacts. T1 relaxation reduction rate T1D% was calculated in the hepatobiliary phase: $T1D\% = (T1N - T1E) / T1N$ [11]. (Some imaging features figures are shown in Figure 1.)

Immunohistochemical analysis

IHC was carried out to detect the protein expression level of TGF- β 1 in HCC specimens. The primary antibody is rabbit monoclonal antibody (EPR21143 /TGF-B1 antibody) used for immunohistochemical staining of all nodular specimens with TGF- β 1, The second antibody is goat anti-mouse/rabbit IgG polymer (SP-9000). Intratumoral specimens were taken from 1-5 sites of the primary lesion to avoid necrosis and bleeding. All specimens were stained with TGF- β 1 immunohistochemistry and the TGF- β 1 positive staining was defined as brown / yellow staining of the cell cytoplasm and membrane. In this study, after hematoxylin reverse staining, the percentage of positive cells for TGF- β 1 was quantified microscopically by two independent examiners. The IHC staining of the TGF- β 1 protein expression was categorized: Grade1 with staining in <10% (-) , Grade 2 with staining in 10%-50% (+) , and Grade3 with staining in \geq 50% (++) for tumor cells [12](Fig. 2).

RT-PCR

Trizol reagent (Invitrogen) were used to extract total RNA from freshly collected specimens in strict accordance with standard operating instructions. RT-PCR was performed to amplify target RNA, during which the following primers were used: TGF- β 1: forward TTGACTTCCGCAAGGACCTC, reverse TCCAGGCTCCAAATGTAGGG. The internal reference primer is GADPH. SYBR primers and miRNA reverse transcription polymerase chain reaction kits are used for polymerase chain reaction amplification. Then Ct value of the sample was obtained according to the analytical dissolution curve and the real-time amplification curve. TGF- β 1 gene expression in HCC tissues relative to that in adjacent tissues was calculated using the following formula: $2^{-\Delta\Delta Ct}$. Where, $\Delta\Delta Ct = \Delta Ct_{ca} - \Delta Ct_{caliv}$, $\Delta Ct_{ca} = Ct_{ca} - Ct_{GADPH}$, $\Delta Ct_{caliv} = \Delta Ct_{caliv} - Ct_{GADPH}$ [13]. The relationship

between TGF- β 1 gene expression and MRI qualitative and quantitative features was analyzed.

Statistical analysis

All statistical analyses were performed using software IBMSPSS23.0(Chicago, IL, USA).Nonparametric tests were performed to assess the differences in the qualitative features of different MRI groups. The difference between the MRI features of multiple groups was determined by the Kruskal-Wallis H test. Spearman rank correlation analysis was used to assess the relationship between quantitative MRI features and gene expression. The variables with statistical differences in univariate analysis were input into the logistic regression model. The screening adopts positive (conditional) method, the exclusion standard is 0.1,the inclusion standard is 0.05. $P < 0.05$ was considered statistically significant.

Results

TGF- β 1 gene expression in HCC

IHC staining for TGF- β 1 was performed in 60 HCC tissues, indicating its protein expression <10%, 10%-50%, and \geq 50% in 27, 14, and 19 cases, respectively. RT-PCR analysis of TGF- β 1 mRNA expression levels was conducted in 54 HCC tissues, of which 15 were positive while 39 were negative for TGF- β 1 gene expression.

Qualitative evaluation of Gd-EOB-DTPA-enhanced MRI features in HCC

Among 60 HCC patients, tumor thrombosis occurred in 11 cases (18.3%), while the majority of the patients (49, 81.7%) had no thrombosis. In T2WI, An inhomogeneous signal intensity was identified in 56 cases, accounting for as high as 93.3%, while homogenous signal intensity was detected in 4 cases (6.7%). In addition, a large proportion of HCC tissues (44/59, 73.3%) showed hypo-intensity in the hepatobiliary phase, and the remaining 16 cases (26.4%) exhibited a heterogeneous signal intensity of both hypo- and hyper-intensity in the hepatobiliary phase. There were 29 cases with complete capsule (48.3%), 13 cases with incomplete capsule (21.7%), and 18 cases without capsule (30.0%). Patterns of the malignant nodules showed smooth single the nodules in 24 cases (40.0%), prominent nodules in 24 cases (40.0%), multiple nodules fusion in 11 cases (18.3%), and infiltrating type in 1 case (1.7%). Characterization of tumor vessels revealed 22 cases (36.7%) with tumor vessels, 38 cases (63.3%) with no vessels, of which 23 cases (38.3%) with hemorrhage, and 37 cases (61.7%) without hemorrhage. Fat-containing malignancies were identified in 4 cases (6.7%) with 56 cases (93.3%) in the absence of intratumoral fat.

Quantitative evaluation of Gd-EOB-DTPA-enhanced MRI features in HCC

The quantitative enhanced MRI features [e.g. tumor size, ADC value , T1 mapping for assessment T1 relaxation reduction] in HCC. The size of 60 HCC lesions ranged from 24.00 to 159.00 mm in diameter (mean, 66.1 mm; SD, 36.85

mm). DWI ($b = 800 \text{ s/mm}^2$) was performed to measure ADC value in 60 HCC cases. The range of ADC was $(0.62\text{--}1.96) \times 10^{-3} \text{ mm}^2/\text{s}$, and its average value was $(0.96 \pm 0.22) \times 10^{-3} \text{ mm}^2/\text{s}$. 22 cases were performed T1 mapping to measure T1 relaxation reduction in HCC. T1D% ranged from 0.21 to 0.64 with an average of 0.42.

Correlation analysis of TGF- β 1 gene expression and enhanced MRI features in HCC

We examined the relationship between TGF- β 1 gene expression and enhanced MRI features in HCC. Spearman rank correlation analysis indicated that there were statistically significant differences in apparent diffusion coefficients among patients with different TGF- β protein expression levels, and there was a slight negative correlation ($p=0.038$, Correlation Coefficient: -0.269). The transcription level of TGF- β 1 gene was significantly different between the hypointense group and heterogeneous signal group in the hepatobiliary phase ($P = 0.009$). Other MR qualitative and quantitative characteristics were not statistically different between the groups (Table 1 -4 ;Fig. 3).

Multivariate logistic regression analysis suggested that the ADC values were not independent risk factors in the protein expression level of TGF- β 1 ($p>0.05$), while binary logistic regression analysis revealed that heterogeneous signal change in the hepatobiliary phase was an

independent risk factors for the TGF- β mRNA expression, and the positive TGF- β expression was higher in the heterogeneous signal group than in the hypointensity group ($P = 0.012$, $OR = 5.333$, $95\%CI: 1.437-19.801$)(Tables 5).

Discussion

The FDA has approved more than 20 small molecule inhibitors and 65 polymetallic oxidase inhibitors for the clinical treatment of cancer, one of which contains TGF- β R[14]. There is substantial evidence that TGF- β 1 is an important cytokine that promotes tumor progression because it induces epithelial-mesenchymal transformation and activates the Wnt pathway.

Previous studies on the correlation between the expression of this molecule and imaging are limited. For example, there are relatively many articles on the correlation analysis of the expression of VEGFR, RAF and other genes with imaging [11, 15]. However, the relationship between the TGF- β 1 gene expression in tumor tissues and MRI features has not been explored by immunohistochemistry and PCR. This study analyzed the preoperative enhanced MRI features and assessed their correlation with TGF- β 1 gene expression level in patients with HCC. The major novel findings were summarized as follows: (1) There was slightly negative correlation between the TGF- β 1 gene expression and enhanced MRI features (ADC values) in HCC; (2) Heterogeneous signal in the hepatobiliary phase on enhanced MRI was independent indicator for high TGF- β 1 expression in HCC; (3) These findings suggested the potential for Gd-EOB-DTPA-enhanced MRI as noninvasive procedure in an evaluation of TGF- β 1 in patients with HCC.

There is growing evidence that differences in gene expression influence responses to targeted therapies, and predicting response rates may be interesting.

In patients with HCC, elevated plasma TGF- β 1 levels are associated with shorter survival [16]. High concentrations of TGF- β 1 are considered as a potential negative prognostic marker in patients with unresectable HCC disease [17]. In hepatocytes, transforming growth factor β induces cell death and epithelial-to-mesenchymal transformation. Several studies have begun to evaluate the safety and efficacy of TGF β -RI kinase inhibitors for advanced HCC. A randomized phase II trial (NCT02178358) is ongoing, with the primary endpoint to evaluate galunisertib plasma concentrations in the presence and absence of sorafenib. Our study demonstrates that MRI features in patients with associated HCC reflect high TGF- β 1 expression, including hepatobiliary heterogeneous signals and ADC values within the tumor, which can be expected to lead to better therapeutic outcomes with the appropriate drugs. Therefore, this study has clinical significance for the selection of appropriate molecular therapy drugs for liver cancer patients in clinical practice.

This study may have some limitations. First, due to the nature of the retrospective study, there may be potential selection bias in the pathological sampling. Secondly, the study population of MRI quantitative characteristics is relatively small, and not all patients can be evaluated by T1 mapping. This requires further study in a larger multicenter cohort. Third, the results of our current study are not completely consistent with the results of IHC and RT-PCR, resulting in inconsistency with the results of correlation analysis for corresponding MRI features, which requires equal sample size or multi-center cooperative analysis in future studies.

Conclusion

Taken together, the findings of this study suggest that the heterogeneous signal in the hepatobiliary phase and the relatively low ADC value of the tumor during enhanced magnetic resonance imaging reflect the high expression of TGF- β 1 in HCC. Considering that TGF- β 1 is a target of small molecule targeted therapy for HCC and is an independent prognostic factor for HCC, enhanced magnetic resonance imaging is a potential approach for prognosis assessment and treatment

selection and efficacy evaluation of targeted therapy in HCC patients. Further research is needed to validate and ultimately improve our care for patients with HCC.

Abbreviations:

△ Ct ca: △ Ct value of carcinoma; caliv: △ Ct value of liver tissue adjacent to carcinoma; GADPH: glyceraldehyde-3-phosphate dehydrogenase

Ethics approval and consent to participate

The research was approved by the Ethic Committee of the First Affiliated Hospital of Guangxi Medical University (Guangxi, China), an equivalent to the Institutional Review Board (IRB). This study was conducted in accordance with ethical guidelines for human research and in accordance with the Health Insurance Portability and Accountability Act (HIPAA). This study confirms that informed consent has been obtained from all subjects or, if subjects are under 18 years of age, from parents and/or legal guardians.

Consent for publication

Not applicable.

Competing interests

The authors (Zhiqing Mo, Liling Long, Hao Ding, Xiaojiao Zhou) declare that they have no competing interests.

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Authors' contributions

The First author: Zhiqingmo, who was responsible for the experimental process, specimen collection and manuscript writing. The corresponding author: Liling Long, who was responsible for reviewing articles and supervising the experiment process. The second author: Hao Ding, responsible for chart processing. The third author : Xiaojiao Zhou, provided experimental guidance and participated in the specimen collection.

Data Availability

The data supporting the conclusions of this study are available from the corresponding author upon a reasonable request.

Conflicts of Interests

The authors declare no competing interests.

Acknowledgement

Not Applicable.

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Figure legends

Figure 1 Gd-EOB-DTPA enhanced MRI features in HCC patients. (A)

Heterogeneous signal intensity of both hypo- and hyper-intensity in the hepatobiliary phase; (B) Hypo-intensity in the hepatobiliary phase; (C) Intratumoral hemorrhage in T1WI ; (D) Intratumoral vessel in the arterial phase by the black arrow ; (E-F) ADC image and its corresponding DWI image with high b value

Figure 2 Immunohistochemical staining of TGF- β 1 in HCC tissues.

Immunohistochemical staining for TGF- β 1 was conducted in 60 HCC tissues. Yellow/brown staining was observed on the nucleus, membrane, and cytoplasm. Positive cells were denoted by the black arrow. (A) Immunohistochemical staining of TGF- β 1 protein expression in histologically normal liver tissues adjacent to HCC; (B) Representative HCC tissues with TGF- β 1 protein expression <10%(-); (C) Representative HCC tissues with TGF- β 1 protein expression 10%-50%(+); (D) Representative HCC tissues with TGF- β 1protein expression \geq 50%(++).

Figure 3 TGF- β 1 mRNA expression levels between the hypointense group and the heterogeneous group in the hepatobiliary phase. The TGF- β 1 mRNA expression was statistically different between the two groups (*P=0.009).

Figures

Figure1

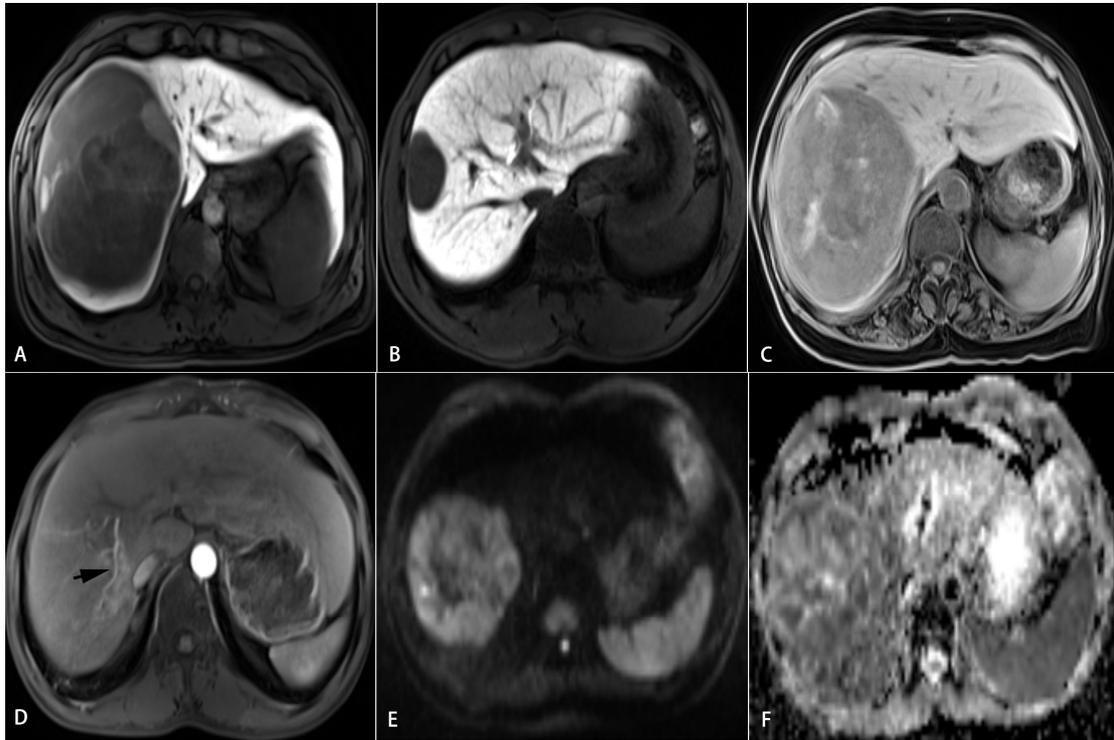


Figure 2

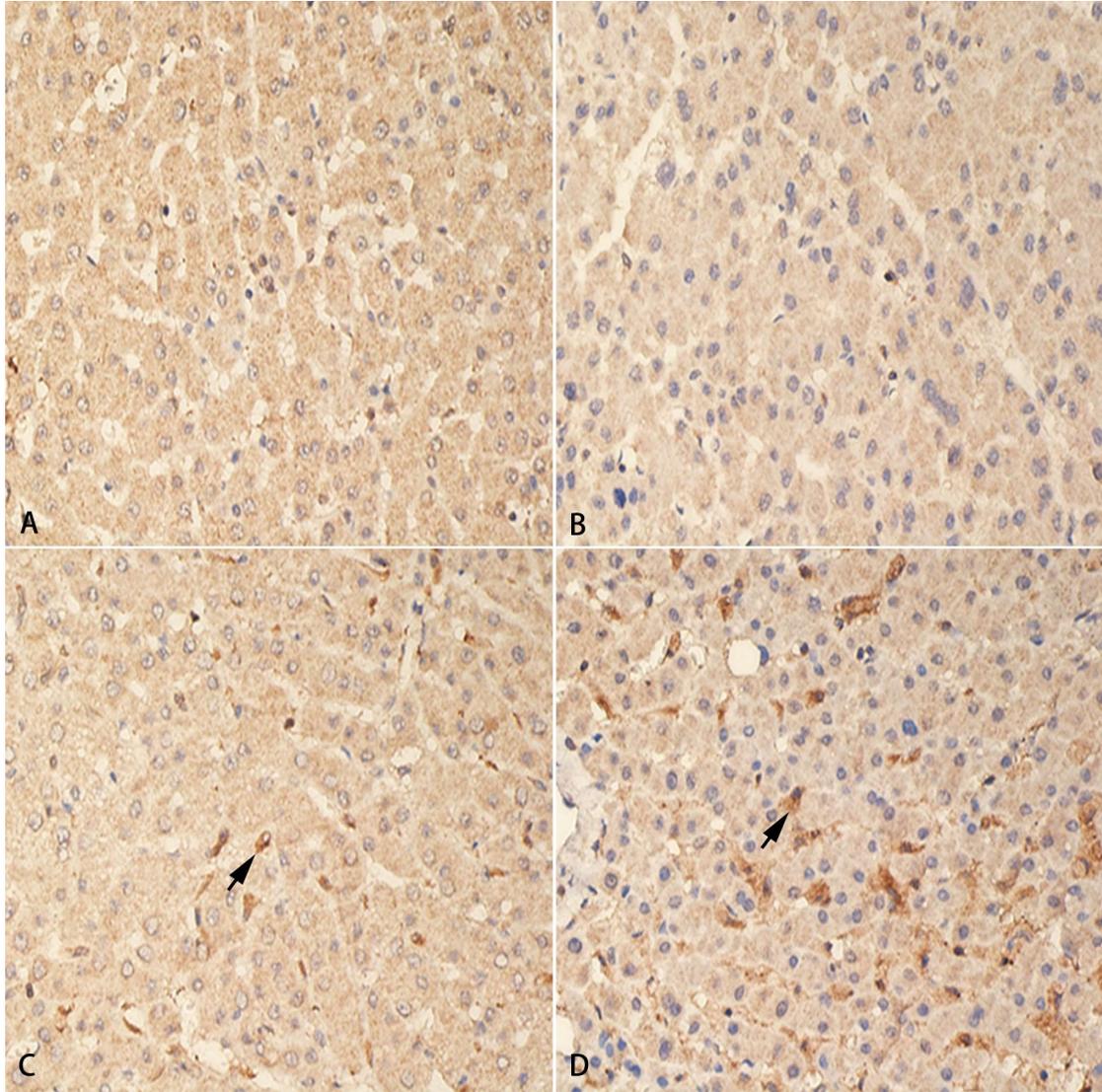


Figure3

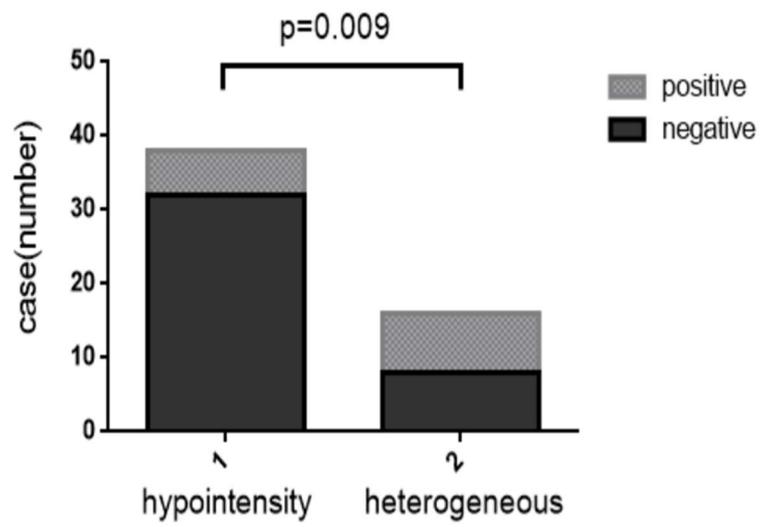


Table1 Comparison between qualitative magnetic resonance imaging(MRI) features and gene TGF- β 1 expression of HCC with immunohistochemical

MRI features (60 cases)	TGF- β 1	
	χ^2	p value
Tumor thrombus		
Yes n=(5)	0.400	0.819
None n=(55)		
T2WI Signal intensity	2.182	0.336
homogeneous n=(4)		
heterogeneous n=(56)		
hepatobiliary phase Signal	0.524	0.769
hypointensity n=(44)		
heterogeneous n=(16)		
Tumor capsule	1.522	0.467
Complete n=(29)		
Incomplete n=(13)		
None n=(18)		
Tumor margins	1.539	0.463
Smooth solitary nodule n=(24)		
Protruding nodule n=(24)		
Fusion of multiple nodules n=(11)		
infiltrative n=(1)		
Intratumoral vascular	0.403	0.817
Yes n=(22)		
None n=(38)		
Intratumoral hemorrhage	0.123	0.942
Yes n=(23)		
None n=(37)		
Fat in tumors	2.642	0.267
Yes n=(4)		
None n=(56)		

Table 2 Correlation between quantitative MRI features and TGF- β 1 expression about immunohistochemical

MRI features	TGF- β 1	
	Correlation coefficient	p value
Maximum diameter (mm) (n= 60)	0.047	0.719
ADC ($\times 10^{-3}$ mm ² /s) (n= 60)	-0.269	0.038
T1D%(n=22)	-0.060	0.789

Table 3 Comparison between qualitative Gd-EOB-DTPA enhanced MRI features and TGF- β 1 mRNA expression level in HCC

MRI features (54 cases)	TGF- β 1	
	χ^2	p value
Tumor thrombus		
Yes n=(8)	0.004	0.949
No n=(46)		
T2WI Signal intensity	1.484	0.223
homogeneous n=(4)		
heterogeneous n=(50)		
hepatobiliary phase Signal	6.735	0.009
hypointense n=(38)		
heterogeneous n=(16)		
Tumor capsule	0.000	0.983
Complete n=(18)		
Incomplete n=(11)		
None n=(25)		
Tumor margins	1.285	0.257
Smooth solitary nodule n=(21)		
Protruding nodule n=(22)		
Fusion of multiple nodules n=(10)		
infiltrative n=(1)		
Intratatumoral vascular	1.183	0.277
Yes n=(36)		
No n=(18)		
Intratatumoral hemorrhage	0.570	0.450
Yes n=(20)		
No n=(34)		
Intratatumoral fat	0.002	0.965
Yes n=(4)		
No n=(50)		

Table 4 Correlation between quantitative MRI features and TGF-β1 mRNA expression level in HCC

MRI features	TGF-β	
	Correlation coefficient	p value
Maximum diameter (mm) (n= 60)	0.094	0.501
ADC ($\times 10^{-3}$ mm ² /s) (n= 60)	-0.169	0.221
T1D%(n=19)	0.145	0.554

Table 5 Logistic regression analysis of signal performance in the hepatobiliary phase associated with TGF-β1 mRNA expression levels in HCC

Variables	p value	OR	95% CI
Hepatobiliary phase Signal	0.001		
Heterogeneous vs hypointense (n=16/38)	0.012	5.333	1.437, 19.801

Figures

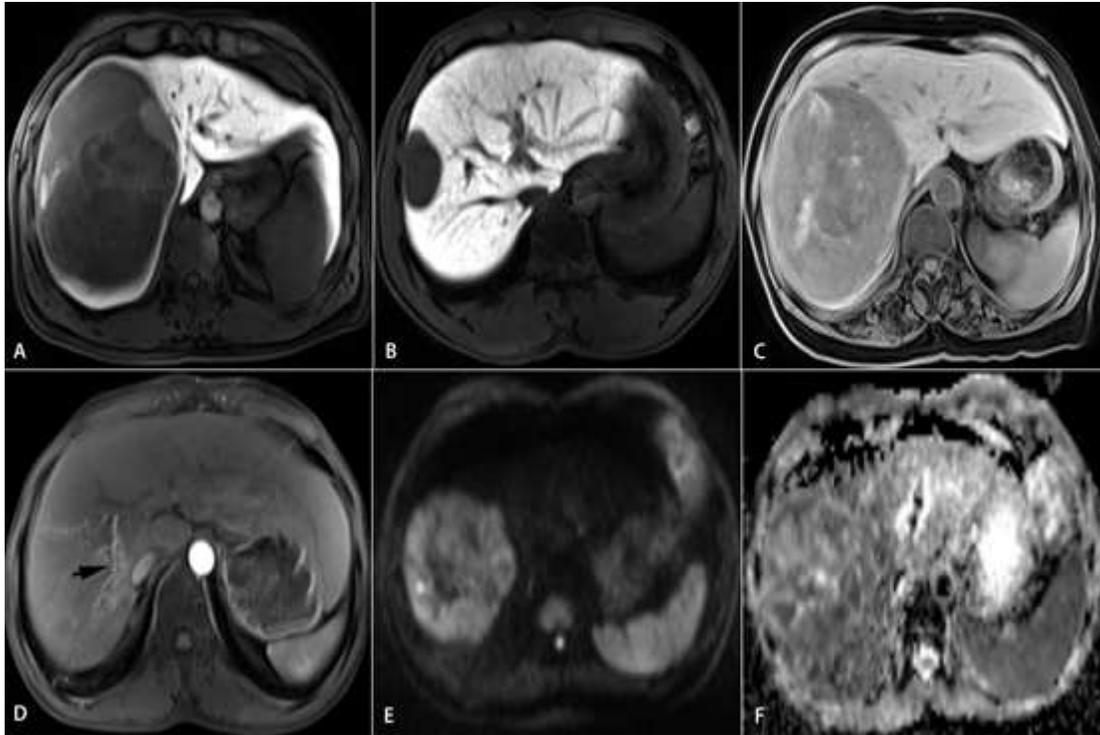


Figure 1

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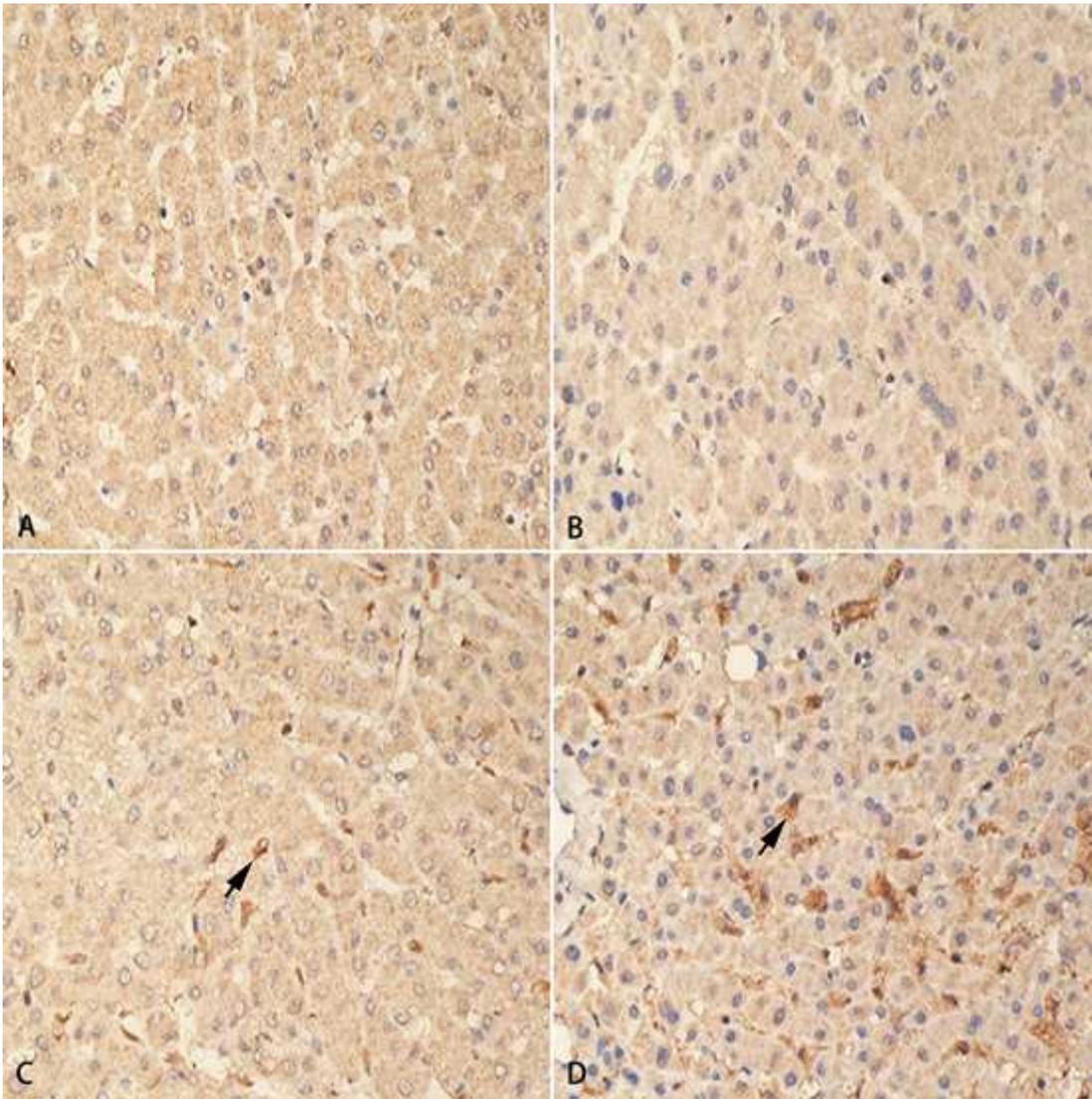


Figure 2

Immunohistochemical staining of TGF- β 1 in HCC tissues. Immunohistochemical staining for TGF- β 1 was conducted in 60 HCC tissues. Yellow/brown staining was observed on the nucleus, membrane, and cytoplasm. Positive cells were denoted by the black arrow. (A) Immunohistochemical staining of TGF- β 1 protein expression in histologically normal liver tissues adjacent to HCC; (B) Representative HCC tissues with TGF- β 1 protein expression <10%(-); (C) Representative HCC tissues with TGF- β 1 protein expression 10%-50%(+); (D) Representative HCC tissues with TGF- β 1 protein expression \geq 50%(++).

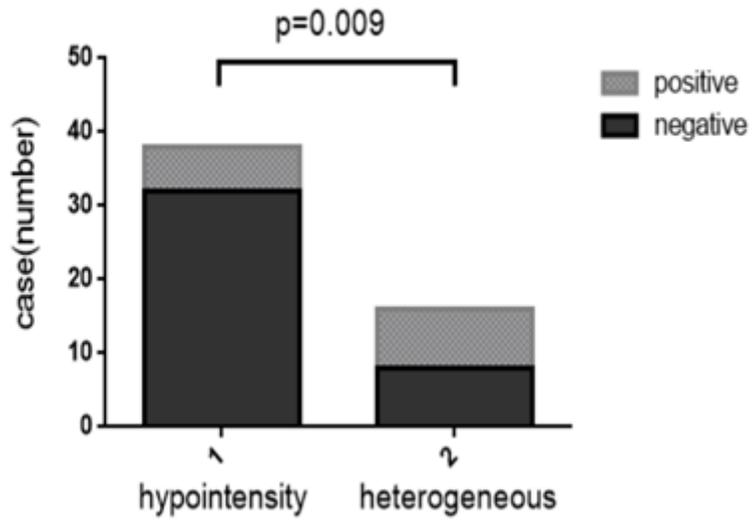


Figure 3

TGF- β 1 mRNA expression levels between the hypointense group and the heterogeneous group in the hepatobiliary phase. The TGF- β 1 mRNA expression was statistically different between the two groups (*P=0.009).