Serum T Cell Immunoglobulin Mucin 3 Predicts Worse Prognosis in Hepatocellular Carcinoma Patients Undergoing Transcatheter Arterial Chemoembolization

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Background: Hepatocellular carcinoma (HCC) is currently a leading cause of cancer-related death, and its prognostic evaluation remains a challenge. We aimed to explore the predictive value of T cell immunoglobulin and mucin 3 (Tim-3) in HCC patients undergoing transcatheter arterial chemoembolization (TACE).

Material/Methods: For the present study, 167 HCC patients who had undergone TACE were retrospectively recruited and randomly stratified into 2 subgroups at a ratio of about 2:1 (training: 109; validation: 58) using EXCEL software. Pretreatment serum was collected from all of these patients, and in a second visit a month after the initial treatment, an additional serum sample was collected from 45 patients in the training set. Tim-3 concentrations were determined by enzyme-linked immunosorbent assay. Kaplan-Meier survival curves analysis, log-rank tests, and Cox proportional hazard regression model were applied to evaluate prognostic significance.

Results: Patients whose cancer progressed after TACE had significantly higher serum Tim-3 in both the training and validation sets (both P<0.05). Kaplan-Meier analysis revealed that patients with elevated serum Tim-3 had significantly shorter time-to-progression and overall survival in both the training and validation sets (both P<0.05). Multivariate Cox regression analysis confirmed that elevated pre-TACE Tim-3 served as a novel, independent indicator for predicting poor prognosis in HCC [progression, hazard ratio (HR) 2.197; 95% confidential interval (CI) 1.408-3.373; P<0.001; death, HR 2.570; 95% CI 1.508-4.378; P=0.001], which was further verified in the validation set (progression P=0.005; death P=0.043). Interestingly, serum Tim-3 retained its prognostic significance in the α-fetoprotein-negative subgroup. Notably, patients with high Tim-3 levels after TACE encountered dismal outcomes.

Conclusions: Serum Tim-3 might be a satisfactory prognostic indicator for HCC patients undergoing TACE.

Keywords: HAVCR2 Protein, Human • Carcinoma, Hepatocellular • Prognosis

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Background

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer-related death worldwide [1]. Surgical resection remains the major approach for treating HCC, but only early HCC patients are suitable for this treatment [2]. Unfortunately, although diagnostic strategy has been updated in recent years, only a minority of HCC patients benefit from early screening, and most HCC patients are diagnosed at an advanced stage. The prognosis of these unresectable HCC patients is relatively poor, evidenced by the median overall survival (OS), which ranges from 7 to 37 months [3]. Conventionally, transcatheter arterial chemoembolization (TACE), an intraarterial catheter-based chemotherapy that selectively delivers drugs to tumor lesions while embolizing the artery, is considered as among the most optimal therapeutic approaches for unresectable HCC patients [4]. However, the response after TACE varies greatly and OS also remains unsatisfactory for these patients, with the approximate 5-year survival rates of TACE ranging from 1-8% [5]. Considering that the treatment is individualized, to improve management efficiency, it is important to identify useful biomarkers for predicting response before applying TACE treatment. However, until now, no reliable serum biomarker has been identified to exert high efficiency in predicting the prognosis of HCC patients who received TACE.

Serum protein markers are considered as the most applicable targets for routine clinical assessments for cancer surveillance due to advantages such as non-invasiveness, lower serum requirement, cost efficiency, and high reproducibility [6]. Escaping immune surveillance is considered as the main driver for cancer progression [7], and secreted immune-related molecules that mediate interactions between the immune system and solid tumors have proven to be ideal circulating biomarkers for predicting prognosis and tumor progression in various types of cancer in HCC [8-10]. Recently, T cell immunoglobulin and mucin domain-3 (Tim-3) was identified as a key regulator and mucin domain-3 (Tim-3) was identified as a key regulator involved in tumor immunological tolerance [11-13]. Functional investigations revealed that Tim-3 expression was closely associated with T cell dysfunction and exhaustion, while the blockade of interaction between Tim-3 and its ligand could effectively restore T cell functions, such as IFN-γ production [14]. Tim-3 is also expressed in a subpopulation of regulatory T cells which exert impressive immunosuppressive capacity in the tumor microenvironment, and this immunosuppression is closely correlated with poor prognosis [15]. Importantly, recent studies have unveiled that Tim-3 can enter peripheral circulation either by active secretion or passive shedding, and that the serum Tim-3 level can also exhibit prognostic value when considering solid tumors [16-18], suggesting that soluble Tim-3 might be a promising biomarker for evaluating prognosis in HCC. However, until now, there has been no evidence demonstrating the clinical relevance of serum Tim-3 in HCC patients receiving TACE.

In light of the above considerations, we speculated that serum Tim-3 might also be a useful biomarker with the potential to predict prognosis in HCC patients undergoing TACE, and we retrospectively recruited 167 HCC patients who had received TACE, and stratified these patients randomly into the 2 subgroups at an approximate ratio of 2: 1 (training set: 109; validation set: 58) to systematically evaluate the value of serum Tim-3.

Material and Methods

Patients and Clinical Samples

First, regarding the sample size of the present study, from April 2014 to December 2015, a total of 167 HCC patients underwent TACE. These patients were retrospectively recruited for our study and randomly stratified into 2 subgroups at an approximate ratio of 2: 1 (training set: 109; validation set: 58) using Microsoft EXCEL software. Briefly, all patients were randomly assigned a number as an individual barcode, then we sorted the patients ascendingly by the barcodes. The top 109 patients were stratified into the training set, while the rest of the patients were stratified into the validation set. Enrollment criteria were as follows: 1) definitive HCC diagnosis, 2) no prior treatment history, 3) received TACE treatments targeting intrahepatic lesions, and 4) availability of complete clinicopathological and followup data. Exclusion criteria: failure to meet any of the above inclusion criteria. For HCC diagnosis, imaging scans, especially enhanced computed tomography (CT) or magnetic resonance imaging (MRI) scans, with typical characteristics of arterial phase enhancement and an alpha-fetoprotein (AFP) test, were taken into consideration according to the American Association for the Study of Liver Disease guidelines. Demographic and clinical data are listed in Table 1. In addition, another 20 healthy donors and 20 resectable HCC patients were also recruited as controls. The present study was performed in accordance with the 1975 Declaration of Helsinki and the Reporting Recommendations for Tumor Marker (REMARK) guidelines. Approval for the use of human subjects was obtained from the research ethics committee of People’s Hospital of Ningxia Hui Autonomous Region, and informed consent was obtained from each enrolled individual. Serum samples were collected 1-2 days before TACE, and centrifuged as soon as they were received at 2000×g for 10 min at 4°C. Afterwards, centrifuged samples were aliquoted and stored at -80°C until serum Tim-3 concentration determination.

Follow-up

Post-treatment surveillance was conducted as previously described [19]. In brief, during the first half year, patients were followed up every month, then followup was conducted every 3 to 4 months. All patients received abdominal ultrasonography and...
Table 1. Correlation between clinicopathologic parameters and sTIM-3 concentration.

| Characteristics | Training cohort | Validation cohort |
|-----------------|-----------------|-------------------|
|                 | sTIM-3          |                   | sTIM-3          |                   |
|                 | Low  | High | P   | Low  | High | P   |
| Gender          |      |      |     |      |      |     |
| Female          | 4    | 6    | 0.488 | 5    | 5    | 0.628 |
| Male            | 51   | 48   |      | 28   | 20   |      |
| Age (y)         |      |      |     |      |      |     |
| ≤50             | 20   | 23   | 0.506 | 20   | 13   | 0.512 |
| >50             | 35   | 31   |      | 13   | 12   |      |
| ALT (U/L)       |      |      |     |      |      |     |
| ≤40             | 41   | 27   | 0.937 | 20   | 12   | 0.339 |
| >40             | 14   | 27   |      | 13   | 13   |      |
| Tumor size (cm) |      |      |     |      |      |     |
| ≤5              | 19   | 12   | 0.008 | 22   | 12   | 0.153 |
| >5              | 36   | 42   |      | 11   | 13   |      |
| PVTT            |      |      |     |      |      |     |
| Absent          | 36   | 36   | 0.894 | 21   | 16   | 0.977 |
| Present         | 19   | 18   |      | 12   | 9    |      |

Serum Tim-3 Concentration Measurement

Serum Tim-3 concentrations were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) using the AbCam proprietary SimpleStep ELISA® technology kit (Human TIM3 ELISA Kit, AbCam, ab231932, Cambridge, USA), according to the manufacturer's instructions. Generally, this was a one-step, single-wash, sandwich ELISA kit designed for the determination of serum or plasma Tim-3 concentration, which was suitable for our present study. First, serum samples were double diluted, and then 50 μL of samples or standards were added into the appropriate wells. Next, 50 μL of antibody cocktail, including capture and detector antibodies, were added into each well containing the samples or standards. The whole plate was then incubated at room temperature for 1 hour, followed by aspiration and washing 3 times with 350 μL 1× Wash Buffer provided in the detection kit. Afterwards, 100 μL of tetramethyl benzidine (TMB) Development Solution was added into each well and incubated in the well for 10 min at room temperature. Finally, 100 μL of Stop Solution was added into each well to stop the reaction and the optimal density of each well was measured by a spectrophotometer (Multiskan Go, Thermo Fisher, USA; purchased from Shanghai, China). Standard curves were established by conducting regression analysis based on all optical density values of the standards. Then, detailed concentrations of serum Tim-3 were calculated by plotting the optical density value of each sample on the standard curve and extrapolating to find the concentration. The operator who performed the experiments was blinded to the serum sample information.

Statistical Analysis

Statistical analysis was conducted using SPSS 26.0 software (IBM, USA; downloaded from the library of Ningxia Medical University in China). Continuous variables were expressed as the mean±standard derivation. First, all grouped data underwent an F-test to determine whether the variances were homogeneous. If the variances were homogeneous, a t test was used to evaluate the significance of differences in data between groups. If variances within groups were not homogeneous, the nonparametric Mann–Whitney test or the Wilcoxon signed-rank test was applied. The prognosis value of serum Tim-3 was analyzed using Kaplan-Meier survival curves accompanied by log-rank tests using PRISM GraphPad 8.0 software (GraphPad Software, San Diego, CA, USA). In brief, patients were stratified into 2 subgroups according to their serum Tim-3 levels, and TTP as well as OS rate were compared between these 2 subgroups to establish survival curves. Log-rank tests were performed at the same time, according to the tutorial included in the GraphPad software. The Cox proportional hazard regression model was used to conduct univariate and multivariate proportional analysis using SPSS software. The cutoff value for serum Tim-3 was set at 4475.10 pg/ml; the median value of serum Tim-3 concentration for all patients in the training set. A value of P<0.05 was considered to be statistically significant. For sample volume estimation, PASS 11.0 software was used and estimation was performed using...
parameters as follows: Power=0.90, Alpha=0.05, P1 Proportion (High progression risk subgroup)=0.9, P2 Proportion (Low progression risk subgroup)=0.55, Group Allocation: Equal. Finally, the estimated number of patients was 29 for each subgroup with a Power of 0.901. Hence, the sample size of both cohorts met the minimum requirement.

## Results

### Serum Tim-3 Was Elevated in HCC Patients Undergoing TACE and Showed No Correlation with Serum AFP

First, serum Tim-3 concentrations among healthy donors, resectable HCC patients, and unresectable HCC patients receiving TACE treatment were compared. Serum Tim-3 concentration in HCC patients was significantly higher than that in healthy donors (mean: 5128.8 for TACE: 3131 for resectable vs 850.9 ng/ml, both \( P<0.05 \)).

More importantly, HCC patients who underwent TACE showed an even higher serum Tim-3 level (significantly higher) than that of the resectable HCC patients (mean: 5128.8 vs 3131.3 ng/ml, \( P<0.05 \), Figure 1A), indicating that serum Tim-3 might be correlated with the severity of HCC progression. Moreover, the correlation between serum Tim-3 and AFP, a widely used serum biomarker for HCC diagnosis as well as prognosis evaluation, was assessed. Serum Tim-3 concentration showed no significant association with serum AFP, in both the training and validation sets (Figure 1B, 1C), suggesting that Tim-3 might serve as a surrogate biomarker that could supplement or complement AFP in the clinical practice of HCC management.

### Higher Serum Tim-3 Concentration Correlated with Poor Prognosis

We further evaluated whether serum Tim-3 was associated with worse prognosis in HCC patients who received TACE. We

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**Figure 1.** Serum Tim-3 distribution and correlation with serum AFP level. (A) Distribution of serum Tim-3 in healthy controls, resectable HCC patients, and unresectable HCC patients receiving TACE treatment. (B) Correlation between serum Tim-3 and AFP in the training set. (C) Correlation between serum Tim-3 and AFP in the validation set. AFP – alpha-fetoprotein; HC – healthy controls; HCC – hepatocellular carcinoma; TACE – transcatheter arterial chemoembolization; Tim-3 – T cell immunoglobulin and mucin domain 3.
found that patients who suffered from progression after primary TACE treatment had significantly higher serum Tim-3 levels than patients without progression, in the training set (Figure 2A). When we stratified patients into 2 subgroups according to the median concentration of serum Tim-3; into the training set as serum Tim-3<sup>high</sup> (>4475.1 pg/ml) and serum Tim-3<sup>low</sup> (<4475.1 pg/ml) subgroups, we found that serum Tim-3<sup>high</sup> patients had higher progression incidence than serum Tim-3<sup>low</sup> patients (98.15% vs 72.73%, Figure 2B). Similarly, patients who died had significantly higher serum Tim-3 levels than patients who lived (Figure 2C), and serum Tim-3<sup>high</sup> patients had higher death incidence than serum Tim-3<sup>low</sup> patients (79.63% vs 40.00%, Figure 2D).

In the validation set, patients who suffered progression or death also had a higher serum Tim-3 level, and serum Tim-3<sup>high</sup> patients showed a higher progression or death rate than serum Tim-3<sup>low</sup> patients, which resembled the results in the training set (Figure 2E-2H).

Higher Serum Tim-3 Concentration Indicated Shorter TTP and OS After TACE

As elevated serum Tim-3 correlated with higher progression and death rates, we further investigated the prognostic value of serum Tim-3 in HCC patients who underwent TACE, via Kaplan-Meier curve analysis. As expected, when stratified according to median value of serum Tim-3 concentration in the training set, we found patients with high serum Tim-3 had significantly shorter TTP in both the training (median TTP 7.0 vs 17.6 months, \( P<0.001 \), Figure 3A) and the validation (median TTP 13.3 vs 22.3 months, \( P=0.010 \), Figure 3B) sets. Moreover, patients with high serum Tim-3 also exhibited significantly shorter OS in both the training (median TTP 23.0 months vs unreached, \( P<0.001 \), Figure 3C) and the validation (median TTP 25.6 vs 29.5 months, \( P=0.014 \), Figure 3D) sets. The above data further indicated the clinical significance of serum Tim-3 in prognosis after TACE treatment.
Pre-TACE Serum Tim-3 Served as an Independent Indicator for TTP and OS in HCC

Next, univariate Cox regression analysis was conducted. In the training set, serum Tim-3 ($P<0.001$), portal vein tumor thrombosis (PVTT) ($P<0.001$), and serum AFP level ($P=0.002$, Figure 4A) showed prognostic significance for tumor progression. Also, all 3 of these parameters showed prognostic significance for OS (serum Tim-3: $P<0.001$; PVTT: $P<0.001$; serum AFP: $P=0.039$; Figure 4B). In the validation set, however, we observed serum Tim-3 as the only parameter with prognostic significance for both TTP ($P=0.013$, Figure 4C) and OS ($P=0.016$, Figure 4D).

Of note, serum Tim-3 was confirmed as an independent indicator for both progression [hazard ratio (HR) 2.197; 95% confidence interval (CI) 1.408-3.373; $P<0.001$; Figure 4E] and death (HR 2.570; 95%; CI 1.508-4.378; $P=0.001$, Figure 4F) in the training set. Supporting these results, in the validation set, serum Tim-3 was also verified as an independent indicator for both progression (HR 2.510; 95%; CI 1.315-4.791; $P=0.005$, Figure 4G) and death (HR 2.007; 95%; CI 1.023-3.939; $P=0.043$, Figure 4H). Together, the above results confirmed pretreatment serum Tim-3 as a novel, independent prognostic marker in patients receiving TACE.

**Figure 3.** Kaplan-Meier curve analysis of serum Tim-3 in HCC patients receiving TACE treatment. (A) Kaplan-Meier curve analysis for progression, in the training set, when patients were stratified into 2 subgroups according to serum Tim-3 levels. (B) Kaplan-Meier curve analysis for overall survival, in the training set, when patients were stratified into 2 subgroups according to serum Tim-3 levels. (C) Kaplan-Meier curve analysis for progression, in the validation set, when patients were stratified into 2 subgroups according to serum Tim-3 levels. (D) Kaplan-Meier curve analysis for overall survival, in the validation set, when patients were stratified into 2 subgroups according to serum Tim-3 levels. Kaplan-Meier analyses were conducted and log-rank tests were performed. HCC – hepatocellular carcinoma; TACE – transcatheter arterial chemoembolization; Tim-3 – T cell immunoglobulin and mucin domain 3.

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Figure 4. Cox regression analysis of serum Tim-3 in HCC patients receiving TACE treatment. (A) Univariate Cox regression analysis for progression, in the training set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (B) Univariate Cox regression analysis for overall survival, in the training set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (C) Univariate Cox regression analysis for progression, in the validation set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (D) Univariate Cox regression analysis for overall survival, in the validation set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (E) Multivariate Cox regression analysis for progression, in the training set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (F) Multivariate Cox regression analysis for overall survival, in the training set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (G) Multivariate Cox regression analysis for progression, in the validation set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. (H) Multivariate Cox regression analysis for overall survival, in the validation set, when the patients were stratified into 2 subgroups according to serum Tim-3 levels. HCC – hepatocellular carcinoma; TACE – transcatheter arterial chemoembolization; Tim-3 – T cell immunoglobulin and mucin domain 3.
Pre-TACE Serum Tim-3 also Exhibited Prognostic Significance in the Low-AFP Subgroup

We further explored prognostic value of pretreatment serum Tim-3 in the low-AFP subgroup. The results indicated that patients with high serum Tim-3 had significantly shorter TTP in both the training (median TTP: 12.0 vs 19.0 months, \( P < 0.001 \), Figure 5A) and validation (median TTP 14.2 vs 22.3 months; \( P = 0.014 \), Figure 5B) sets. In addition, patients with high serum Tim-3 also exhibited significantly shorter OS, in both the training (median TTP 24.0 months vs unreached, \( P = 0.002 \), Figure 5C) and validation (median TTP 26.1 vs 39.5 months, \( P = 0.003 \), Figure 5D) sets. The above data demonstrated that serum Tim-3 level also exerts significant prognosis prediction value in the low-progression-risk subgroup.

Prognostic Value of Dynamic Alteration of Serum Tim-3 in HCC Patients who Received TACE

We further explored the dynamic changes in serum Tim-3 during the peri-TACE period (before and 1 month after TACE) in 45 patients in the training set. Unfortunately, we did not observe any significant decrease in serum Tim-3 when measured 1 month after TACE, according to paired \( t \) test (data not shown). Therefore, we assessed whether the dynamic changes in the positive states before and after TACE shed influence on prognosis evaluation. These 45 patients were further stratified into 4 subgroups based on peri-TACE serum Tim-3 levels: Group I, high for both pretreatment and post-treatment; Group II, pretreatment high and post-treatment low; Group III, pretreatment low and post-treatment high; Group IV, low for both pretreatment and post-treatment.
low and post-treatment high; and Group IV, remained low after TACE. Interestingly, we found that patients with persistent, high serum Tim-3 had significantly shorter TTP and lower OS than patients who showed a pattern of decreased serum Tim-3 (TTP: $P=0.009$, Figure 6A; OS: $P=0.005$, Figure 6B). Moreover, patients who showed an increased serum Tim-3 pattern had significantly shorter TTP and lower OS than patients with persistent low serum Tim-3 (TTP: $P=0.012$, Figure 6A; OS: $P=0.034$, Figure 6B). However, no statistical significance was observed between the other subgroups. Collectively, our data demonstrated that reduced serum Tim-3 level might be a promising indicator for better prognosis, whereas increased alteration pattern might reflect a worse prognosis.

**Discussion**

Predicting prognosis after TACE via baseline parameters has been a long-term problem that puzzles clinical decision-makers. Identification of useful baseline parameters for accurately predicting treatment response might facilitate personalized therapy for patients with advanced-stage HCC. In the present study, we report that pretreatment serum Tim-3, an easily tested target via ELISA assays, was significantly elevated in HCC patients who then encountered adverse outcomes such as rapid progression or shorter OS after TACE treatment. Importantly, our data demonstrated that pre-TACE serum Tim-3 served as a novel, independent indicator of poor prognosis in HCC patients undergoing TACE. Moreover, monitoring of dynamic changes in serum Tim-3 during the peri-TACE period might provide accurate information for predicting long-term prognosis. Among the many types of circulating parameters, non-protein circulating biomarkers have been identified—for example, cell-free DNAs or cell-free RNAs. However, circulating protein biomarkers remain the most applicable detection target for routine clinical use, because generally such detection methods are non-invasive, require a relatively low volume of specimen, are not dependent on an operator with high expertise, are cost-efficient, have high reproducibility, and have extremely simple sample-pretreatment protocol (for example, no need for extraction or prior purification procedures) [6]. Hence, application of serum Tim-3 detection might be a useful approach for improving HCC management in the future.

Tim-3, firstly identified in 2002, is a well-established immune checkpoint molecule that exerts strong inhibitory effects on anti-cancer immunity by repressing cytotoxic T cell proliferation, and reducing the production of effector cytokines of these T cells [20-22]. As a cell membrane surface molecule, Tim-3 is likely to fall off from the membrane surface due to cell migration or rapid metabolism, and then enter the circulation where it can be detected. As evidence for this, recently, it was also identified as a circulating biomarker involved in the progression of several diseases. For example, increased circulating Tim-3 has been reported to be correlated with the progression of sepsis [23]. Moreover, circulating Tim-3 concentration was also shown to be associated with severe graft-versus-host disease [24] and cutaneous systemic sclerosis [25]. Interestingly, elevated circulating Tim-3 concentration correlated significantly with non-relapse mortality and OS in patients who received...
allogeneic hematopoietic cell transplantation [26]. More importantly, in cancerous situations, circulating Tim-3 has been reported to be elevated in patients with colorectal cancer and osteosarcoma [27,28]. In HCC, increased serum Tim-3 level has been identified as a novel prognostic indicator for patients undergoing curative resection [18]. In middle- or advanced-stage HCC patients who received TACE, serum Tim-3 level was elevated when HCC stage escalated [17]. However, the prognostic value of serum Tim-3 remained elusive in HCC patients receiving TACE. Here, we provided evidence that detection of pre-treatment serum Tim-3 might provide useful information for predicting poor prognosis after TACE. Our data was in accordance with previous studies, and further extended the clinical relevance of serum Tim-3, demonstrating that determination of serum Tim-3 might screen out who might benefit from TACE, and those who have elevated pre-treatment serum Tim-3 might benefit from more frequent surveillance and followup after TACE.

In clinical practice, AFP remains the most popular serum biomarker for HCC [29]. Unfortunately, about 30 to 40% of HCC patients showed low serum AFP (<400 ng/ml) when diagnosed, indicating a strong need for novel circulating biomarkers for these AFP-low patients [30-32]. Therefore, the predictive performance of serum Tim-3 in the low-AFP subgroup is of great potential benefit. Our data showed that detection of serum Tim-3 could further stratify AFP-low patients into 2 subgroups, termed serum Tim-3 low or serum Tim-3 high, and more importantly, individuals in the serum Tim-3 low subgroup also had significantly shorter TTP and OS than patients in the serum Tim-3 high subgroup. Our findings demonstrated that serum Tim-3 is a promising prognostic biomarker for HCC, especially for patients with low serum AFP. Detection of pre-TACE serum Tim-3 can help the clinicians to identify patients at high risk of progression who need more careful surveillance or additional therapy.

There were several limitations of our present study. First, due to lack of sufficient samples collected from healthy donors, we could barely construct the normal range and optimal cut-off value for serum Tim-3 to discriminate HCC patients from healthy individuals, a project being concurrently undertaken in our center. Second, we also could not determine the precise origin of serum Tim-3 in HCC patients. Since Tim-3 could be expressed on the surface of several types of cells, the origin of serum Tim-3 could be confirmed by conducting immunohistochemistry staining in HCC tissues. However, we failed to obtain resected HCC tissues in the present study. Nonetheless, we could still observe that patients with decreased serum Tim-3 levels had better prognoses, suggesting a close association between serum Tim-3 and tumor burden. We will further evaluate this association in our future work. Finally, most HCC patients in China have a background of hepatitis B virus infection, which differs greatly from the patient population in the United States, Europe, or Japan, according to previous studies [6]. Hence, the prognostic value of serum Tim-3 needs further verification in patients with different etiologies and clinical histories.

Conclusions

To the best of our knowledge, the present study is the first investigation to demonstrate the prognostic predictive value of serum Tim-3 in HCC patients who have received TACE treatment. Further investigation into the function of serum Tim-3 in regulating systemic immune cells, especially with regard to CD8+ T cell exhaustion, might provide a novel insight into the mechanism of HCC immunotolerance and immune evasion by HCC cells. Collectively, determination of serum Tim-3 concentration during the TACE treatment period might be useful for improving prognosis of HCC patients.

Declaration of Figures’ Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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