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ORIGINAL ARTICLE

C1GALT1C1/COSMC is a novel prognostic biomarker for hepatocellular carcinoma

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KEYWORDS
COSMC;
Hepatocellular carcinoma;
Predictive model;
Prognosis

Summary
Background/aims: The aim of this study is to explore the effects of COSMC on the prognosis of hepatocellular carcinoma (HCC), and establish a novel model with improved predictive capacity.
Methods: Ninety-two patients diagnosed with HCC from 2006 to 2010 in our hospital were recruited to analyze the correlation between COSMC expression and prognosis. Cellular experiments were performed to verify the anti-tumor effects of COSMC. A predictive model was established based on the risk factors from multiple COX regression analysis. After validation, the novel model was compared with the conventional model in terms of capacity of predicting the prognosis.
Results: The expression of COSMC was lower in tumor tissues than in normal tissues and inhibited HCC migration in cells. Besides the expression of COSMC was significantly negatively correlated with overall survival (OS) in HCC, regression analysis showed that COSMC expression, vascular invasion, and TNM stage were prognostic risk factors. Our novel model comprising these three elements was established and validated. Besides the good fit of the calibration curves, a higher concordance index (C-index) for OS ($P = 0.011$) as well as better decision curve analysis (DCA) and survival curves for both disease-free survival (DFS) and OS suggested the superiority of this novel model compared with conventional TNM staging in predicting the prognosis of HCC patients.

Abbreviations: HCC, hepatocellular carcinoma; OS, overall survival; index, concordance index; DCA, decision curve analysis; TMN, tumor-node-metastasis; DFS, disease-free survival; TCGA, the cancer genome atlas; siRNA, small interfering RNA; HRP, horseradish peroxidase; IHC, immunohistochemistry; EMT, epithelial-mesenchymal transition.

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Conclusions: We established a novel model by integrating the expression of COSMC, vascular invasion, and TNM stage, and found that it was better able to predict survival in patients with HCC compared with conventional TNM staging.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide, accounting for more than half a million deaths annually [1,2]. Given its increasing incidence, HCC has become a major health burden globally, although several effective therapies have been developed in recent decades, including surgical resection, transcatheter arterial embolization chemoembolization, radiofrequency ablation, and molecular targeted treatment [3—5]. The 5-year overall survival (OS) rate of patients with HCC is less than 10%; thus, improvements in prognosis are urgently needed for better management of this disease [1].

Despite the fact that several clinical characteristics have been identified as prognostic markers for HCC [6,7], the most commonly used model for predicting survival is still the tumor-node-metastasis (TNM) classification system [8]. In addition, several nomogram-based models have been established to improve the predictive capacity [9—11]. Nevertheless, it is difficult to accurately predict the prognosis of individual patients due to tumor heterogeneity.

It is well known that the Tn antigen (GalNAcα1-O-Ser/Thr) is associated with the progression and poor prognosis of various cancers [12—14]. C1GALT1C1/COSMC, which is located in the endoplasmic reticulum, is the only known molecular chaperone of T-synthase. T-synthase initiates the biosynthesis of O-glycans by transferring N-Acetylgalactosamine (GalNAc) to serine threonine residues on proteins to produce Tn antigen in many types of epithelial cancers [15,16]. In this setting, oncogenesis derived from the overexpression of COSMC has been documented in numerous cancers, including pancreatic, colon, and breast cancers [12—14]. Thus, the potential exists to develop novel treatments via immunotherapy approaches that target Tn antigen or associated proteins, thereby providing a novel platform for diagnosing patients based on individual tumor profiles [17]. Nonetheless, the role of COSMC in HCC has not been completely elucidated. The results of this study showed that COSMC has the potential to be a valuable predictive biomarker in patients with HCC.

Materials and methods

Study design

To investigate the role of COSMC in HCC, we searched clinical databases (http://www.cbioportal.org; gepia.cancer-pku.cn) [18] and analyzed the expression of COSMC as well as the correlation between COSMC and tumor histological grade and survival. To investigate the role of COSMC in the carcinogenesis of HCC, several cellular experiments were performed to evaluate cell proliferation, apoptosis, and migration.

To validate the correlation of COSMC with prognosis, 92 informed patients diagnosed with pathologically confirmed HCC between 2006 and 2010 were recruited into this study (as the development cohort). This study, conforming to the provisions of the Declaration of Helsinki, was approved by the Ethical Committee for Human Subjects at Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University (Hangzhou, China). Demographic and pathological characteristics including age, sex, alpha fetal protein, underlying hepatic disease, tumor differentiation grade, vascular invasion, and TNM stage were collected retrospectively. The endpoints were disease-free survival (DFS) and OS. DFS was defined as the interval from the date of surgery until the detection of tumor recurrence, and OS was defined as the interval from the date of surgery until death. Then, univariate Cox regression analysis was conducted to identify potentially prognostic elements. Potential variables ($P < 0.05$) were entered into the subsequent multivariate Cox regression analysis, and a novel predictive model was established based on the identified risk factors. To evaluate the generalization of this model, an external cohort from The Cancer Genome Atlas (TCGA) database was used as the validation cohort. After excluding cases that lacked results from follow-up or complete information on prognostic factors, demographic and pathological characteristics of the enrolled cases in the validation cohort ($n = 250$) were collected. In the development and validation cohorts, the calibrations were performed with 500 bootstrap samples using R software (version 3.3.3) with the ''rms'' package. Furthermore, the concordance index (C-index), decision curve analysis (DCA), and stratified survival analysis were adopted to evaluate the predictive capacity [19].

In vitro cell culture/maintenance

The HepG2 human HCC cell line (RRID: CVCL_0027) was purchased from the American Type Culture Collection (Manassas, VA). The HLE cell line was a gift from Professor Ji Junfang Lab from the Institute of Biology, Zhejiang University. All of the cell lines were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (v/v), penicillin (25 units/mL), streptomycin (25 g/mL), 1% L-glutamine, and 10% FBS. Both cell lines were cultured in a 5% (v/v) CO_2 humidified incubator at 37 °C.

COSMC overexpression and cell transfection

HepG2 cells were transfected with the pWPI-COSMC overexpression plasmid or empty vector as a control using MegaTran 1.0 transfection reagent (Origene, Rockville, MD). For small interfering RNA (siRNA) (si-COSMC: 5′-GTAAGAGCTTTCGAGTATA-3′) transfection, cells were seeded overnight in 6-well plates ($3—5 \times 10^5$ cells/well)

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and transfected using Lipofectamine™ RNAiMAX Reagent (Invitrogen). A negative control (siRNA) supplied by the manufacturer was used as a control.

Cell viability, apoptosis, transwell, and would healing assays

Cell lines were seeded in a 96-well plate with three replicate wells and allowed to incubate for 72 h. After incubation, cell viability was assessed using the Cell Counting Kit-8 protocol (Dojindo, Kumamoto, Japan). The percentage of apoptotic cells was determined by flow cytometry according to the manufacturer's instructions (Dojindo).

For the transwell assay, cells were trypsinized and resuspended in corresponding medium containing 1% FBS at a density of 1×10^6 cells/mL. Cell suspension (100 μL) was added to the upper chamber of a transwell (Corning, Corning, NY) Medium (600 μL) containing 10% FBS was placed in the lower chamber. After a 48 h incubation at 37 °C, the cells remaining in the upper chamber were carefully removed by a cotton swab. The side facing the lower chamber was stained with 0.05% crystal violet, and the attached cells were counted under a light microscope.

For the wound healing assay, 1×10^6 cells/mL were seeded on a 6-well plate with three replicate wells. A cell-free gap was created by scratching a confluent monolayer with a pipette tip. After a 72 h incubation, the degree of healing was detected using ImagePro Premier (Media Cybernetics, Rockville, MD). The migration capacity was evaluated using the following migration index: migration index (%) = (gap at 72 h)/(gap at baseline) × 100.

Real-time quantitative PCR

Total RNAs were isolated using Trizol reagent (Invitrogen), from the frozen tissue of primary tumors and adjacent non-tumor liver tissues. Total RNA (1 μg) was subjected to reverse transcription using Superscript III transcriptase (Invitrogen). Real-time quantitative PCR was conducted using the Bio-Rad CFX96 System (Bio-Rad Laboratories, Hercules, CA) with SYBR green to determine the mRNA expression levels of genes of interest. Expression levels were normalized against the expression of GAPDH mRNA. The primer sequences for COSMC were: forward primer (5′ to 3′): AGCGAGACCAACGAGAGAAC; reverse primer (5′ to 3′): TGCTTCCAAGCATCACACCC.

Western blot analysis

Frozen tissues were treated with RIPA buffer, and proteins (60 μg) were separated on 10—12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and then electrotransferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). After blocking the membranes, they were incubated overnight at 4 °C with appropriate dilutions of specific primary antibodies. After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific, Rochester, NY). COSMC and α-tubulin primary antibodies were purchased from Abgent (AP22217a; San Diego, CA) and Abcam (ab6160; Cambridge, MA), respectively.

Immunohistochemical staining

Immunohistochemistry (IHC) was used to evaluate the expression of COSMC in 92 HCC samples. Tissues were fixed in 10% (v/v) formaldehyde in phosphate-buffered saline, embedded in paraffin, cut into 5-μm sections, and used for IHC staining. To enhance antigen exposure, the slides were treated with 1× armour citrate at 98 °C for 10 min for antigen retrieval. The slides were incubated with endogenous peroxidase blocking solution, followed by incubation with primary antibody at 4 °C overnight. After rinsing with Tris-buffered saline, the slides were incubated for 45 min with biotin-conjugated secondary antibody, washed, and then incubated with HRP-conjugated streptavidin. Freshly prepared 3,3'-diaminobenzidine (Zymed, South San Francisco, CA) was used as a substrate to detect HRP. Positive cells (%) were calculated as the number of immunopositive cells × 100 divided by the total number of cells/field in four random fields at a 400× magnification. The expression of COSMC was assessed semi-quantitatively as follows: < 5%, negative; 5—30%, weak positive; and ≥ 30%, positive. Negative, weakly positive expression, and positive expression were defined as low, middle, and high expression, respectively. According to the expression of COSMC assessed by two independent pathologists, stratified survival analysis was performed.

Statistical analyses

For cytological experiments, data were presented as the mean with standard deviation and compared with the Student's *t*-test. The clinical variables were presented as numbers with proportions. Correspondingly, the chi-square test or Fisher's exact test was used to compare categorical data as appropriate. Survival data were described using Kaplan—Meier curves and compared using the log-rank test. Statistical significance was considered when two-tailed *P* values were less than 0.05. Statistical analyses were performed using SPSS version 22.0 for Windows (IBM Corporation, Armonk, NY).

Results

COSMC expression in HCC

As shown in Fig. 1A, the expression of COSMC was significantly lower in tumor tissues than in paired or non-paired non-tumor tissues. Meanwhile, a negative correlation was demonstrated between COSMC expression and histologically differentiated HCC (Fig. 1B). Further analysis of our clinical samples (25 paired tissues at the mRNA level and 6 paired tissues at the protein level) confirmed that the expression of COSMC was lower in HCC than in paired adjacent tissues (Fig. 1C). For prognosis, high COSMC expression was significantly associated with longer DFS and OS, as determined from clinical data from the TCGA database (Fig. 1D). Taken

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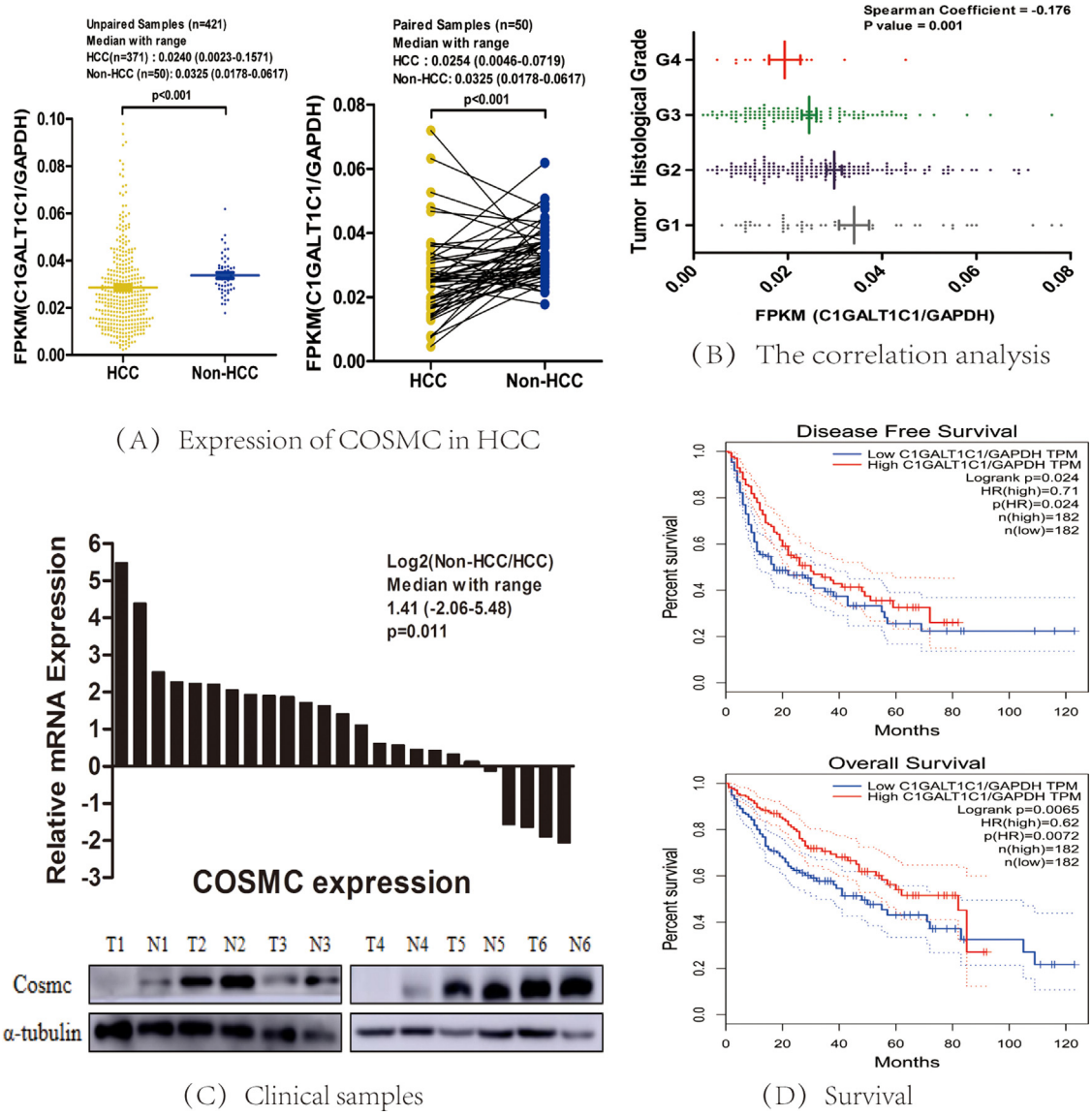

Figure 1 Clinical data of COSMC. (A) The expression of COSMC in TCGA database; (B) The correlation of COSMC expression and tumor histologic grade; (A) and (B) are downloaded from www.cbioportal.org; (C) Expression of COSMC in clinical samples at mRNA and protein levels; (D) Survival analysis dependent on the expression of COSMC, downloaded from gepia.cancer-pku.cn.

together, these results suggest that COSMC overexpression inhibits HCC carcinogenesis.

COSMC inhibits the migration of HCC

To further investigate the role of COSMC in HCC carcinogenesis, several cellular assays were performed. Given the expression of COSMC in different cell lines was measured (Supplement Fig. 1A), the lowest and highest expression cell lines (HepG2 and HLE, respectively) were used for our studies. There were no significant differences in cell proliferation when COSMC was overexpressed in HepG2 cells ($P = 0.156$) or knocked down by siRNA in HLE cells ($P = 0.188$) (Supplement Fig. 1B and 1C). Likewise, the percentage of later apoptosis were comparable after manipulating COSMC in HepG2 ($P = 0.321$) and HLE ($P = 0.052$) (Supplement Fig. 1D and 1E). However, the wound healing assay revealed that increased COSMC expression in HepG2 cells was associated with significantly slower wound closure ($P = 0.017$; Fig. 2A), whereas reduced COSMC expression in HLE cells resulted in significantly faster wound closure ($P = 0.024$; Fig. 2A). The transwell assay demonstrated that COSMC overexpressing cells were less invasive than control cells ($P = 0.025$; Fig. 2B), whereas si-COSMC cells were more invasive than si-NC cells ($P = 0.003$; Fig. 2B). Accordingly, western blot analyses showed that COSMC expression significantly increased expression of epithelial-mesenchymal transition (EMT)-related genes such as E-cadherin and decreased the expression of mesenchymal markers such as vimentin and N-cadherin (Fig. 2C).

Clinical data analysis of HCC patients

As previously mentioned, 92 patients with HCC were recruited to this study (Table 1). Based on assessment of

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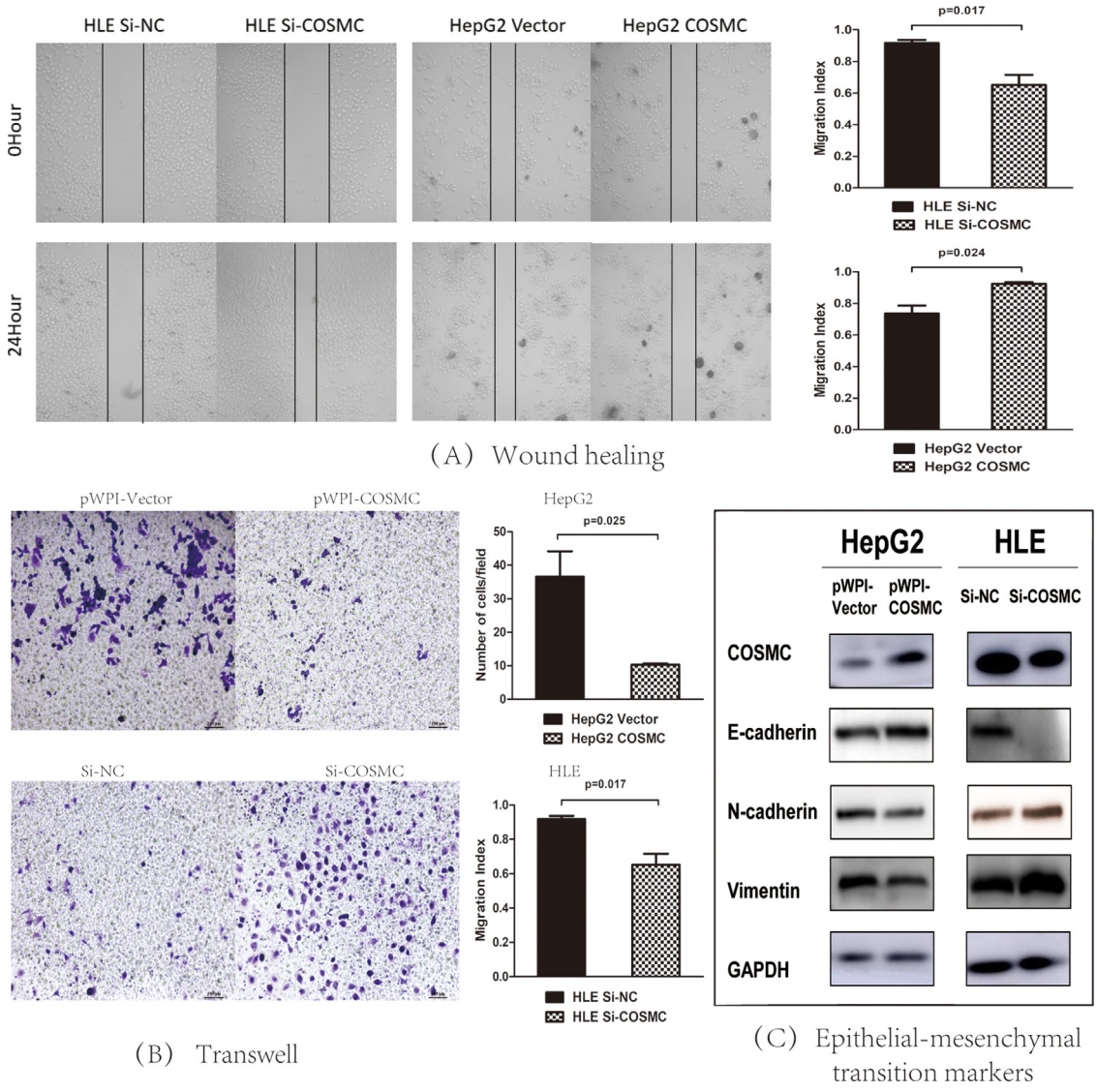

Figure 2 COSMC inhibits the metastasis of HCC. (A) The wound healing assay indicated that increased expression of COSMC in HepG2 cells was associated with significantly slower wound closure, whereas reduced COSMC expression in HLE cells resulted in significantly faster wound closure. Upper panel, representative images of wound healing assay; Lower panel, quantification of the healed gap; (B) Chamber-transwell invasion assays showed that overexpression of COSMC decreased the cell invasion in all HepG2 and down-regulation of COSMC increased the cell invasion in HLE. Left panel, representative images of the chamber-transwell migration assays; Right panel, quantification of the migration cells. The invaded cells were counted in 4 randomly chosen microscopic fields (100 ×) of each experiment and pooled.

COSMC expression by IHC (Fig. 3A), the patients were divided into three subgroups: high, middle, and low expression groups. No significant differences were found among these subgroups regarding their demographic and pathological characteristics (Supplement Table 1). Pearson's correlation analysis revealed that the coefficients between COSMC expression and DFS and OS were −0.187 and −0.305, respectively ($P = 0.075$ and $P = 0.003$). Contrary to expectations, no significant correlation was found between COSMC expression and DFS, possibly due to the limited number of cases in this study (Fig. 3B). Survival analysis showed that lower COSMC expression was associated with unfavorable OS ($P = 0.0174$), and the low-expression subgroup presented a trend towards inferior DFS (Fig. 3C).

Establishment of a novel prognostic model

Because the above-mentioned results showed that COSMC expression may be a prognostic factor for survival, we attempted to establish a novel model with improved predictive capacity. Initially, univariate followed by multivariate regression analysis was performed in the development cohort, in which COSMC expression, vascular invasion, and TNM stage were found to be relevant factors for survival (Table 2). In addition, the expression of COSMC was demonstrated to be a prognostic factor for both DFS and OS, whereas TNM stage III/IV predicted a poor outcome in OS (hazard ratio [HR], 4.452; $P = 0.008$) and vascular invasion predicted an unfavorable DFS (HR, 3.481; $P = 0.001$), respec-

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Table 1 Clinicopathological characteristics of internal and external cohorts. Data are presented as number with percentage. TNM stage is according to the AJCC 7th edition. In external cohort, cirrhosis is defined as Ishak score 5 or 6.

	Internal cohort ($n = 92$)		External cohort ($n = 250$)	
	Number	Percentage (%)	Number	Percentage (%)
Age, years				
≥ 50	47	51.1	50	20.0
< 50	45	48.9	200	80.0
Gender				
Male	87	94.6	172	68.8
Female	5	5.4	78	31.2
Serum α-fetal protein, ng/ml				
≥ 400	35	38.0	171	68.4
< 400	57	62.0	47	18.8
Missing data	—	—	32	12.8
Cirrhosis				
Present	49	53.2	65	26.0
Absent	43	46.8	103	41.2
Missing data	—	—	82	32.8
Tumor grade				
Grade 1—2	47	51.1	150	60.0
Grade 3—4	45	48.9	100	40.0
Vascular invasion				
Present	13	14.1	84	33.6
Absent	79	85.9	166	66.4
TNM stage				
I	71	77.2	138	55.2
II	12	13.0	63	25.2
III	6	6.5	46	18.4
IV	3	3.3	3	1.2

T: tumor tissue; N: non-tumor tissue. Data are presented as number with percentage. TNM stage is according to the AJCC 7th edition. In external cohort, cirrhosis is defined as Ishak score 5 or 6.

tively. Subsequently, a risk stratification was defined as follows: vascular invasion (absent, score of 0; present, score of 1 for OS and 2 for DFS), expression of COSMC (high, score of 0; middle, score of 1; low, score of 2), and TNM stage (stages I—II, score of 0; stages III—IV, score of 2). The novel model was classified into three levels: low risk, score ≤ 1; medium risk, score 2—3; and high risk, score ≥ 4 (Table 3).

To determine the generalization of this model, an external validation cohort ($n = 250$) was selected from the TCGA database. The clinical features of this cohort are summarized in Table 1. For calibration, the plots for the probability of postoperative 1-, 3-, and 5-year survival are presented in Supplement Fig. 2, which fit well with the actual survival rates in both the development and validation cohorts.

Comparison of the novel model and TNM staging

To evaluate clinical applicability, a comparison between our novel model and traditional TNM staging was performed. In terms of discriminatory capacity, the C-indexes of different models are presented in Fig. 4. In the development cohort, the C-index of the novel model for OS was 0.670 ± 0.042, which was significantly higher than the 0.563 ± 0.044 calculated for OS by TNM staging ($P = 0.011$). No significant difference was found in the C-index for DFS between groups. However, the results in the validation cohort showed that the predictive capacity of our novel model was significantly more accurate than that of TNM stage for both DFS and OS ($P < 0.001$ for both; Fig. 4A). Given that the proposed novel model demonstrated superior predictive capacity in terms of the C-index, DCA was conducted to ascertain its applicability in clinical settings. The novel model had a better net benefit with a wider range of threshold probabilities for both DFS and OS (Fig. 4B). These results further indicated the improved predictive performance at higher threshold probabilities of the novel model, as well as its net benefit levels.

The stratified survival analysis of various models is shown in Fig. 5. In the development cohort, the number of patients was 83 and 9 in TNM stages I/II and III/IV, respectively, whereas in the validation cohort, 201 and 49 patients were in TNM stages I/II and III/IV. For the novel model, the number of patients in low-, middle-, and high-risk groups was 34, 43, and 15, respectively, in the development cohort. Correspondingly, 113, 78, and 59 patients belonged to low-, middle-, and high-risk groups, respectively. How well the development and validation cohorts conformed between the novel model and TNM stage was compared. The novel model displayed a good fit, while the discrepancy between the development and validation groups for TNM stage was clear (Fig. 5). In summary, these findings demonstrate that the

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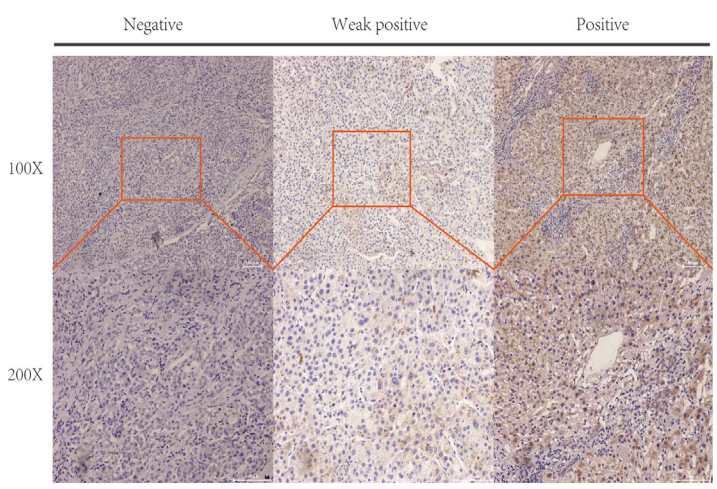
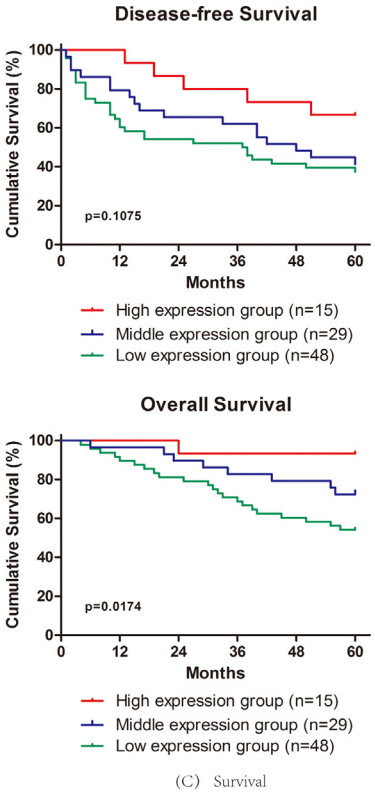


(A) Expression of COSMC in HCC patients

Pearson's correlation

	COSMC expression	P-value
Disease-free survival	-0.187	0.075
Overall survival	-0.305	0.003

(B) Relationship of COSMC and survival



(C) Survival

Figure 3 Negative correlation of COSMC expression and survival. (A) Detection of COSMC expression by immunohistochemistry (IHC); (B) Pearson's correlation between COSMC expression and DFS/OS; (C) Survival analysis dependent on the expression of COSMC. Low, middle and high expression groups are defined as negative, weak positive and positive groups in IHC stain. DFS: disease-free survival; OS: overall survival.

novel model more accurately predicted survival than traditional TNM staging.

Discussion

Given the high heterogeneity of HCC, a prognostic model integrating genetic information and clinical characteristics, thereby improving predictive accuracy has long been of interest. A handful of studies have revealed that COMSC promotes tumor occurrence, invasion, and metastasis in various cancers [12—14]. However, the role of COSMC in HCC is not fully understood. Thus, in this study, we attempted to clarify the function of COSMC in HCC.

First, the expression of COSMC in HCC was evaluated by searching online databases. The data showed that COSMC expression was up-regulated in paired or non-paired non-tumor tissues compared to HCC tissues. The high expression of COSMC in cell lines had attenuated invasiveness. Further validation of clinical samples from our hospital confirmed the low expression of COSMC in HCC. Additionally, a strongly negative relationship between COSMC expression and vascular invasion as well as tumor differentiation suggested its anti-neoplastic properties in HCC [12—14,17]. Subsequent survival analysis confirmed that lower expression of COSMC was associated with shorter survival. These data indicate that COSMC can serve as a novel prognostic biomarker for HCC.

Considering the need for an improved clinical prediction model for HCC, we constructed a novel model based on the identified risk factors from multiple regression, namely COSMC expression, vascular invasion, and TNM stage. Following establishment of the novel model, its generalization was validated using an external cohort from TCGA database. Given the higher C-index, the better net benefit with a wider range of threshold probabilities in DCA, and the superior fit in the hierarchical survival curve, strong evidence indicated that COSMC expression could improve the capacity of predicting the prognosis of HCC.

Since it was first reported in 2002 [20], COSMC has served as a molecular target for regulating Tn antigen, sialyl-Tn antigen (sTn) antigen, and T antigen by adjusting the folding of T-synthase to promote malignant behavior in various cancers [21,22]. In clinical settings, sTn-specific IgGs derived from anti-sTn vaccines inducing antibody-mediated tumor protection has been documented in several publications [23,24]. Immunotherapies targeting the sTn/T antigen may reduce metastasis and prolong survival. Alternatively, MUC-1 (a carrier protein of T antigen and Tn antigen) was shown to strongly enhance the immune response by stimulating T-cell-specific responses to inhibitors of carcinogenesis in mouse models [25].

Although COSMC can promote the progression of cancer in a Tn antigen-dependent manner, the well-known PI3K/AKT and MEK/ERK signaling pathways are also involved in COSMC-derived tumor growth and metastasis [14,26—28]. However,

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Table 2 Univariate and multivariate analysis for survival in development cohort.

	Disease-free survival						Overall survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age, years												
< 50		Reference						Reference				
≥ 50	0.815	0.473—1.404	0.461				1.097	0.540—2.225	0.798			
Gender												
Female		Reference						Reference				
Male	1.412	0.603—3.307	0.427				0.911	0.350—−2.373	0.849			
AFP, ng/ml												
< 400		Reference						Reference				
≥ 400	0.878	0.491—1.571	0.662				1.149	0.557—2.367	0.707			
Cirrhosis												
Absent		Reference						Reference				
Present	1.281	0.739—2.220	0.378				1.154	0.568—2.341	0.692			
Tumor grade												
Grade 1—2		Reference						Reference				
Grade 3—4	1.212	0.700—2.094	0.494				1.319	0.650—2.677	0.443			
Vascular invasion												
Absent		Reference			Reference			Reference			Reference	
Present	3.525	1.795—6.921	< 0.001	3.481	1.638—7.399	0.001	2.333	1.004—5.425	0.049	1.548	0.571—4.194	0.379
TNM stage												
I—II		Reference			Reference			Reference			Reference	
III—IV	2.811	1.262—6.262	0.011	2.421	0.997—5.881	0.051	3.723	1.514—9.154	0.004	4.524	1.489—13.742	0.008
Expression of COSMC												
Low expression		Reference			Reference			Reference			Reference	
Middle expression	0.807	0.445—1.464	0.480	0.605	0.325—1.129	0.114	0.517	0.230—1.162	0.110	0.379	0.161—0.891	0.026
High expression	0.375	0.145—0.964	0.042	0.317	0.122—0.826	0.019	0.114	0.015—0.847	0.034	0.101	0.013—0.761	0.026

HR: hazard ratio; CI: confidence interval; AFP: α-fetal protein; TNM stage is according to the AJCC 7th edition.

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Table 3 Definition of novel model.

Risk factor	Value	OS score	DFS score	Total scores	Risk stratification
Vascular invasion	Absent	0	0	0–1	Low risk
	Present	1	2	2	Medium risk
Expression of COSMC	High expression	0	0	≥ 3	High risk
	Middle expression	1	1		
	Low expression	2	2		
TNM stage	I–II	0	0		
	III + IV	2	2		

TNM stage is according to the AJCC 7th edition. OS: overall survival; DFS: disease-free survival.

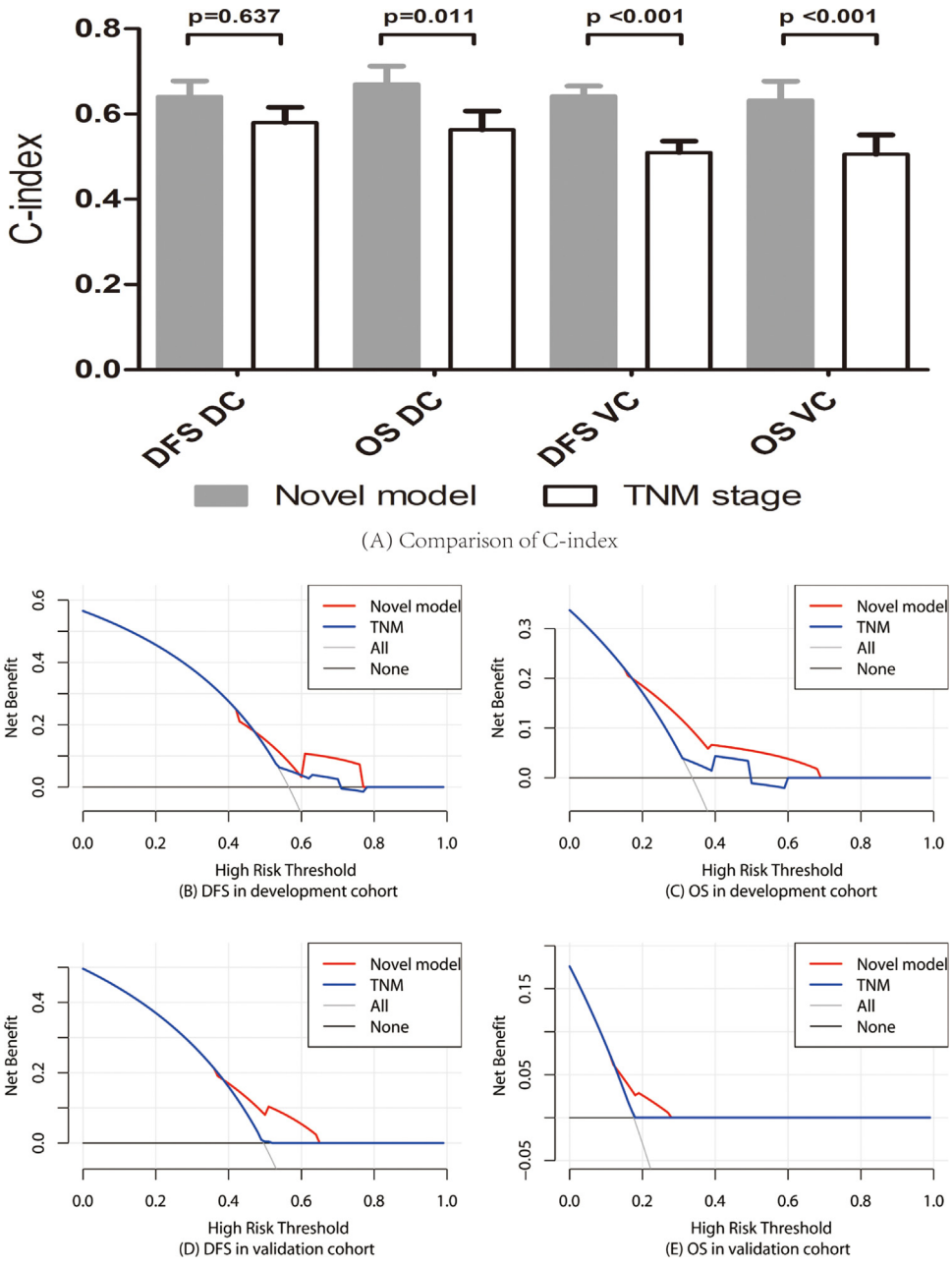

Figure 4 Comparison between novel model and TNM stage. (A) C-index between novel model and TNM stage in development and validation cohorts; (B) decision curve analysis between novel model and TNM stage in development and validation cohorts. DFS: disease-free survival; OS: overall survival; DC: development cohort; VC: validation cohort.

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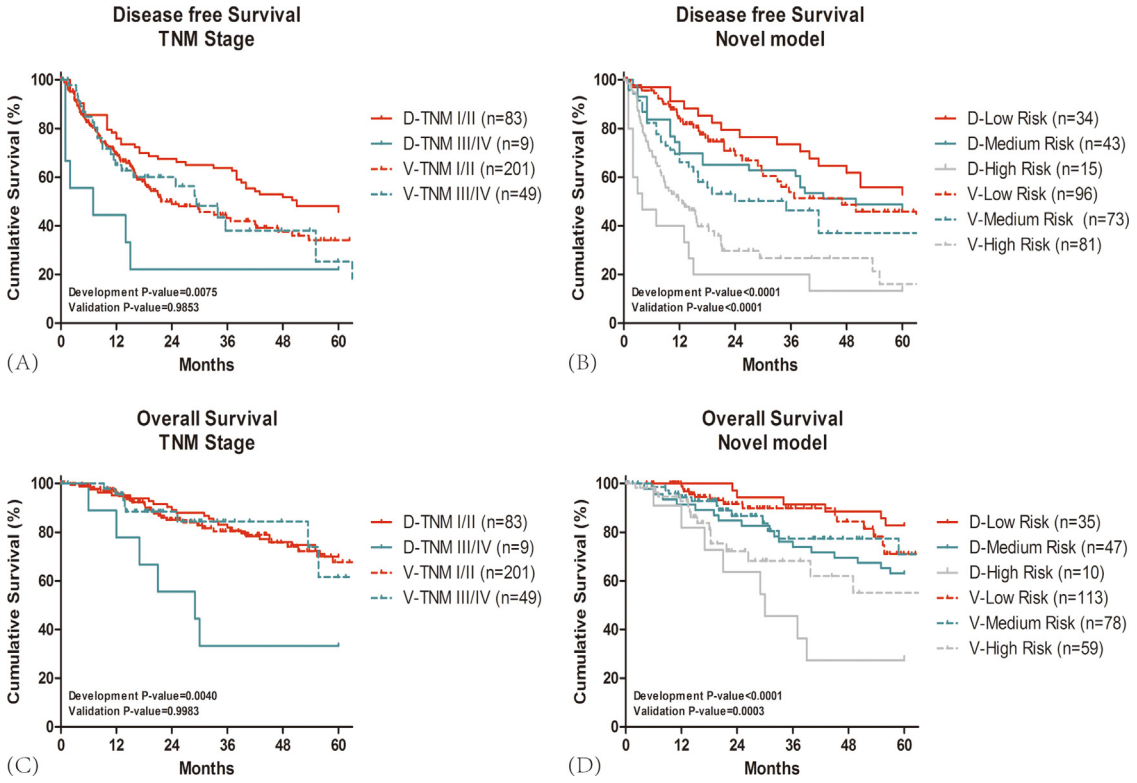

Figure 5 Survival of novel model and TNM stage. (A) DFS in TNM stage; (B) DFS in novel model; (C) OS in TNM stage; (D) OS in novel model.

considering the heterogeneity of tumors and organ specificity, the role of COSMC in HCC has not been fully clarified. Our cytological experiments indicated that COSMC could inhibit the metastasis of HCC through the EMT pathway, but the mechanism remains to be further clarified. In future investigations, we will explore how COSMC inhibit carcinogenesis in HCC in a non-Tn antigen-dependent pathway, which has the potential to be a therapeutic target.

In conclusion, the results of this study demonstrated that COSMC has anti-neoplastic properties in HCC. In addition, we established a novel model by integrating the expression of COSMC, vascular invasion, and TNM stage, which has better predictive capacity than conventional TNM staging. Because prognosis is the most important clinical factor for patients and their relatives, this novel model could be a useful instrument for facilitating communication during the informed consent process.

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Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.dx.org/10.1016/j.clinre.2019.07.017.

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