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Nomogram based on CMTM6 expression and clinical characteristics to predict postoperative overall survival in patients with hepatocellular carcinoma

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Summary. Background. The purpose of this study was to investigate the expression of CMTM6 in HCC tissues and its prognostic value, and to try to develop a nomogram prognostic model based on CMTM6.

Methods. In this retrospective study, immunohisto-chemical (IHC) staining was performed in 178 patients who underwent radical hepatectomy in the same surgical team. R software was used to construct the nomogram model. The Bootstrap sampling method was used for internal validation.

Results. CMTM6 is significantly expressed in HCC tissues and is closely associated with decreased overall survival (OS). PVTT (HR=6.2, 95% CI: 3.06 12.6, P<0.001), CMTM6 (HR=2.30, 95% CI: 1.27 4.0, P=0.006) and MVI (HR=10.8, 95% CI: 4.19-27.6, P<0.001) were independent predictors of OS. The nomogram combined with CMTM6, PVTT and MVI was more predictive than the traditional TNM scoring system, and the prediction effects of 1-year and 3-year OS were accurate.

Conclusions. The prognosis of a patient may be predicted using high levels of CMTM6 expression in HCC tissues, and the nomogram model including CMTM6 expression has the best predictive ability.

Key words: CMTM6, HCC, Prognosis, Nomogram, Model

Introduction

Primary liver cancer, the sixth most frequent type of cancer, is the third most frequent cause of cancer-related mortality worldwide (Bray et al., 2018). Among them, HCC accounts for 75-85% of all histological subtypes of primary liver cancer (Cadier et al., 2017). Hepatitis B

virus infection is the most common risk factor in China (Yu et al., 2020; Campbell et al., 2021). At present, although progress has been made in treatment, including advanced surgery, liver transplantation, ablation therapy (radiofrequency ablation, microwave ablation, cryoablation, etc.) and molecular targeted drugs (chitosan nanoparticles, lutein, etc.), less than 35% of liver cancer patients survive for five years (Li et al., 2014; Cheng et al., 2017; Forner et al., 2018; Kulik and El-Serag, 2019; Zhao et al., 2022; Jiang et al., 2021). Therefore, it is crucial to thoroughly investigate the incidence and development process of HCC from a molecular perspective and look for highly sensitive molecular potential markers in order to aid in early diagnosis, therapeutic therapy, and prognosis prediction of HCC.

The genes CKLF and CMTM 1-8 form a novel gene family known as Chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing family (CMTMs). A member of the CMTM family named CMTM6 (Han et al., 2001, 2003) resides on chromosome 3p22.3 and is 55% similar to CMTM4 in terms of amino acid composition. The coding product of CMTMT6 is mainly distributed in the cell membrane and cytoplasm. It is a type 3 transmembrane protein with MARVEL domain (Li et al., 2020b) and contains at least three transmembrane helices, which are mainly involved in regulating the transport of transmembrane and secreted proteins. Recent studies identified that CMTM6 was co-localized with PD-L1 and acted as a critical regulator for the maintenance of PD-L1 expression in various cancer types (Burr et al., 2017; Yaseen et al., 2022). When PD-L1 was internalized, CMTM6 could successfully retract it to the cell surface, expand the protein's half-life, boost the immune system's ability to inhibit the cell, and avoid immune detection. Additionally, it was found that high-grade malignant gliomas had increased CMTM6 expression, suggesting that this finding might be connected to the poor prognosis of glioma patients (Guan et al., 2018). It is worth mentioning that a nomogram can accurately predict the probability of an outcome event by

combining multivariate analysis and integrating multiple predictors, and it can directly calculate the value of a variable using graphs. It has been used in the medical field for a long time, and its prediction of tumor prognosis is more accurate than the traditional scoring and staging system (Weiser et al., 2008; Cai et al., 2020). Consequently, we investigated CMTM6's function in HCC and assessed its worth in the detection and management of HCC. In order to examine the association between CMTM6 expression and clinicopathological characteristics and patient, the level of CMTM6 expression in HCC and surrounding tissues was initially assessed in this study. The development of a prognostic model for HCC patients followed, with the aim of providing a reference for the diagnosis, therapy, and prognosis of HCC.

Materials and methods

Clinical data

Between June 2018 and August 2021, Cangzhou People's Hospital identified and treated 207 patients with HCC. These patients were included in the retrospective analysis. Medical records were used to extract information on gender, age, HBsAg (Hepatitis B surface antigen) status, AFP (alpha fetoprotein) expression, and pathological data (such as tumor size,

PVTT (portal vein tumor thrombus), MVI (micro-vascular invasion), and TNM (Tumor-Node-Metastasis) stage. All patients received routine follow-up care. The best option was outpatient care. The two main extra follow-up approaches were by phone and email. OS, which is measured as the period from operation to death or the last visit, was the follow-up outcome. The follow-up period ended in April 2022. The inclusion criteria: (a) Both sexes, aged>18 years; (b) Initial treatment; (c) Postoperative pathology confirmed HCC; (d) All patients had complete clinical data chart; (e) The distance of adjacent tissues from the cancer tissue ≥2cm, and no tumor cell infiltration. Exclusion criteria: (a) previous history of other primary cancer diseases; (b) Patients who have received chemoradiotherapy or immunotherapy; (c) Medical history of immune system or traumatic infection. Following screening, 178 patients were chosen to participate in the trial. In Fig. 1, the selecting procedure is depicted. The hospital's ethics committee gave the study its approval. Phone calls were used to get consent.

Experimental reagents

CMTM6 antibody (NBP1-31183) and rabbit anti-human monoclonal antibody were purchased from Novus (USA). DAB immunohistochemical assay kit, return blue staining solution and hematoxylin staining solution were purchased from Roche Diagnostic Products (Shanghai) Co., Ltd.

Immunohistochemical staining

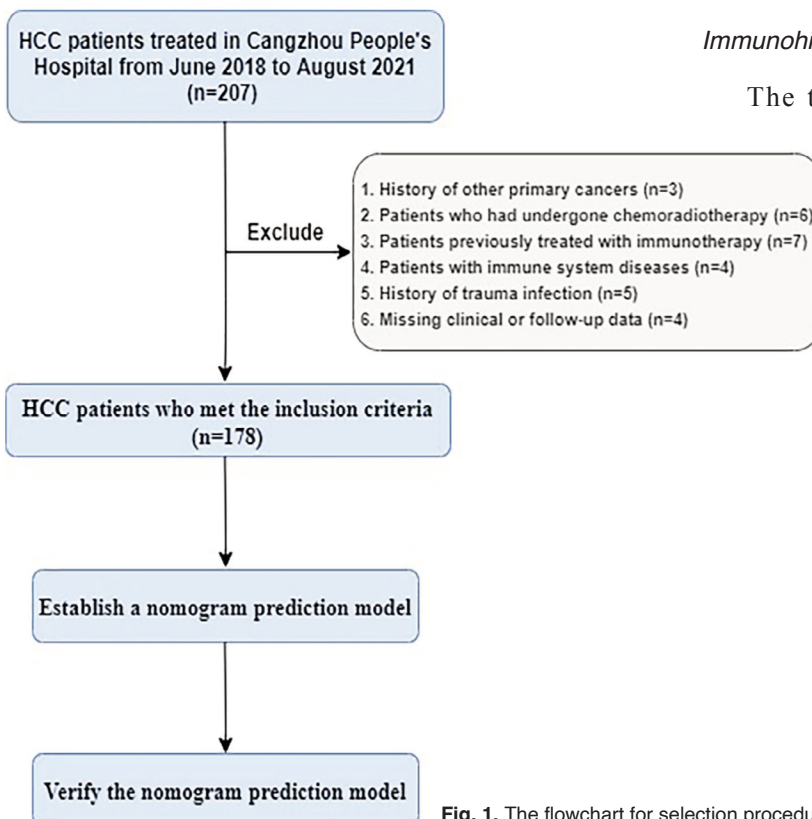The tissue samples were cut into 4-μm-thick

Fig. 1. The flowchart for selection procedure of HCC patients.

sections, dewaxed in xylene, and then rehydrated using a gradient ethanol solution. The sample was soaked in EDTA buffer to recover the antigen. After washing with PBS (phosphate buffered solution) 0.3% H_2O_2 was used to block endogenous peroxidase. Human CMTM6 Rabbit polyclonal Antibody (1:1000 dilution; CMTM6 antibody) was applied to the slices and incubated overnight in a 4°C refrigerator. On the second day, the slices were washed with PBS and added with mouse/rabbit universal secondary antibody, incubated at 37°C for 30 min, color developed with 3,3'-diaminoben-zidine (DAB), and restained with Mayer hematoxylin. Each tissue section was dehydrated by gradient alcohol and covered with cover glass.

The staining results were determined by two senior pathologists from Cangzhou People's Hospital under double-blind conditions. Five fields of high magnification (×200) were randomly selected, and the appearance of brown-yellow granules in the cytoplasm was considered as positive staining for CMTM6. IHC intensity was divided into 4 grades according to the intensity of cell brown: 0 for no staining, 1 for light color, 2 for intermediate color, and 3 for dark brown. The proportion of positive cells was scored as follows: 0 for 0-5%, 1 for 5-25%, 2 for 25-50%, 3 for 50-75%, and 4 for >75%. The final score is the product of staining intensity and the percentage of positive cell scores, and the mean H-score was included in the statistics. In this study, the cut-off value was set as 50. H-score Score >50 was considered as high expression, and H-score <50 was considered as low expression.

Statistical analysis

The data was processed using SPSS 26.0 and the R language 4.0.1 (R, Austria). The sample measurement data are expressed as mean standard deviation if they adhere to the principles of normal distribution; otherwise, they are expressed as median and quartile. Rates or percentages are used to characterize data from enumerations. Kaplan-Meier survival curves and log-rank tests were utilized to examine survival differences across groups. The Cox regression model was used to evaluate the prognostic factors, and variables with a P<0.05 were included and subjected to additional analysis. The Forest plot and Nomogram were created using forestploter and the rms package of the R language based on the findings of the Cox multivariate analysis. The prognostic effects were compared using the C-index and ROC, the accuracy of the prediction model was assessed using calibration curves, and the clinical value of all systems and indicators was assessed using DCA curves. The C-index was calculated by the function of rcorrcens of the Hmisc package of R language. The fluctuation range of C-index is 0.5~1.0, and the larger the value, the more perfect the prediction model. The survival ROC package was used to draw ROC curves. The calibration curve was drawn by the calibrate function of the rms package. The closer the curve is to a

line with slope 1, the higher the accuracy of the prediction model. Through 1000 iterations of Bootstrap repeated sampling, the nomogram model's prediction performance was confirmed. Statistics were deemed significant at P<0.05.

Results

Expression of CMTM6 gene in HCC tissues

IHC was used to identify the expression and subcellular location of the CMTM6 protein in HCC and adjacent tissues. CMTM6 protein immunostaining (Fig. 2) was primarily restricted to the cytoplasm and membrane

Table 1. Relationship between CMTM6 expression and clinico-pathological variables.

Clinicopathological feature	N	CMTM6		χ^2
		High expression	Low expression	
Age				
≤60	104	72	32	0.18
>60	74	49	25	
Sex				
Female	50	36	14	0.517
Male	128	85	43	
Tumor size				
≤5cm	87	63	24	1.539
>5cm	91	58	33	
Stage of T				
T1	88	59	29	8.675
T2	36	20	18	
T3	46	38	8	
T4	6	4	2	
Distant metastasis				
M0	113	66	47	13.021
M1	65	55	10	
LNM				
N0	143	86	57	0.087
N1	35	22	13	
TNM Stage				
I	88	52	36	1.888
II	37	26	11	
III	53	30	23	
HBsAg				
positive	75	37	38	6.986
negative	103	71	32	
AFP				
positive	66	32	34	6.532
negative	112	76	36	
PVTT				
Yes	78	37	41	10.198
No	100	71	29	
MVI				
No	41	21	20	1.996
Yes	137	87	50	

LNM, lymph node metastasis; PVTT: portal vein tumor thrombus; MVI: microvascular invasion; AFP: alpha fetoprotein; TNM: Tumor-Node-Metastasis; HBsAg: Hepatitis B surface antigen.

of HCC cells. The expression rate of CMTM6 in HCC tissues was substantially higher than that in surrounding tissues. [68% (121/178) vs 23% (41/178); P<0.001]. The results showed that the expression level of the CMTM6 protein in HCC tissues was much greater than in the corresponding adjacent tissues.

Relationship between the clinicopathological characteristics of HCC patients and the expression of CMTM6

According to the level of CMTM6 expression the patients were divided into two groups: 121 had high CMTM6 expression, and 57 had low CMTM6 expression. 178 HCC patients' clinicopathological features, including age, sex, HBsAg, AFP, tumor size,

PVTT, MVI, and tumor TNM stage, are shown in Table 1. The expression of CMTM6 was Correlated with stage of T, Distant metastasis, PVTT, and AFP expression (χ^2=8.675, 13.021, 10.198, 6.532, P<0.05). There was no association with other clinicopathological features of HCC patients (P>0.05).

Relationship between HCC patients' prognosis and CMTM6 expression

Based on the median level of CMTM6 expression, HCC patients were divided into high and low CMTM6 expression groups, and their survival was evaluated using a Kaplan-Meier survival analysis. Shorter OS in patients with high CMTM6 expression compared to low CMTM6 expression patients suggests that high CMTM6
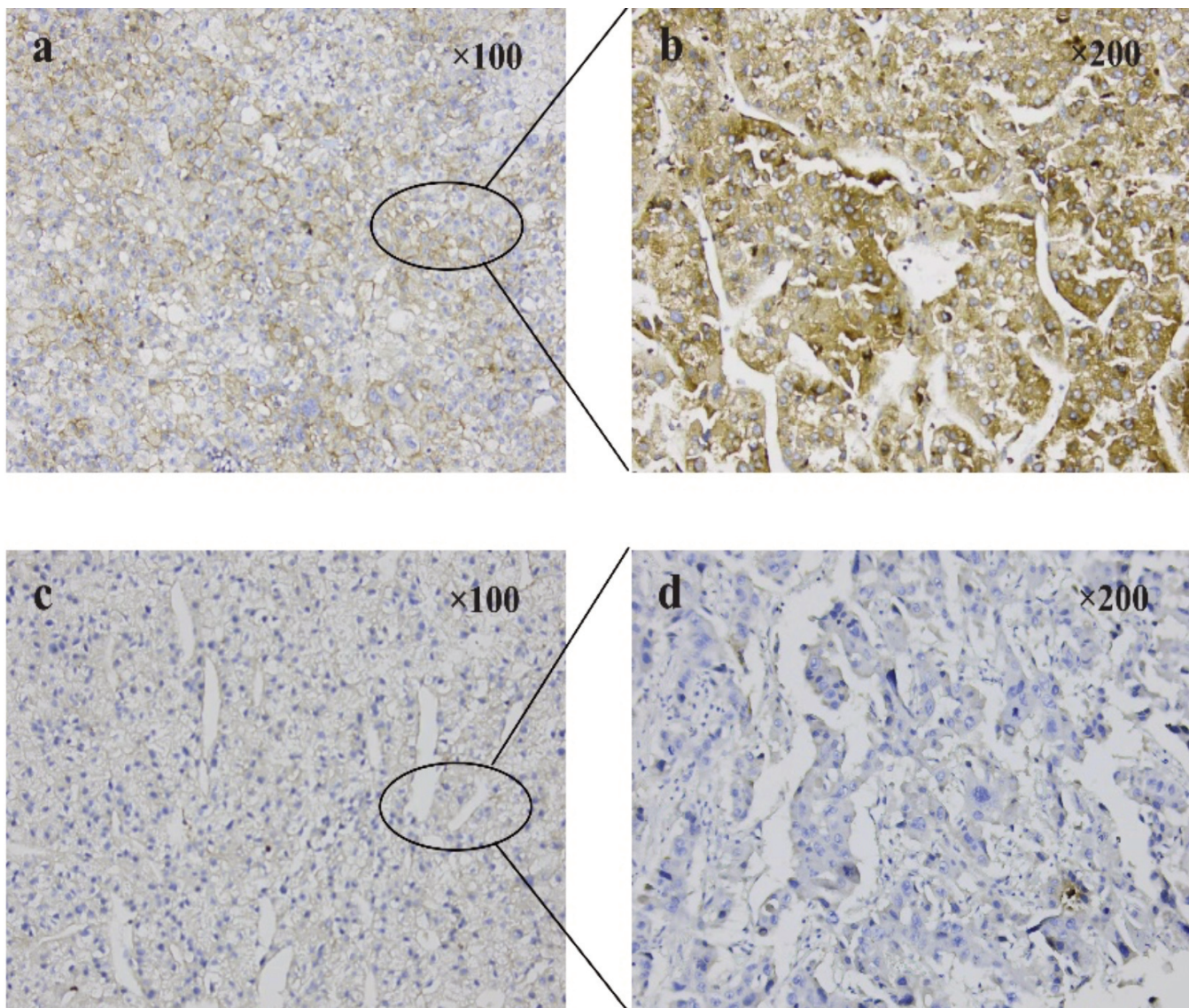

Fig. 2. Expression of CMTM6 in HCC **(a, b)** and paracancer tissues **(c, d)**. a, c, x 100; b, d, x 200.

expression is associated with a poor prognosis (P=0.03; Fig. 3). To ascertain the effect of CMTM6 and clinically relevant factors on OS in HCC patients, prognostic variables were screened using both univariate and multivariate Cox regression models. Finally, it was shown that PVTT (HR=6.2, 95%CI: 3.06-12.6, P<0.001), CMTM6 (HR=2.30, 95%CI: 1.27-4.0, P=0.006) and MVI (HR=10.8, 95%CI: 4.19-27.6, P<0.001) could be classified as independent risk factors of HCC patients with poor prognosis (Table 2, Fig. 4).

Construction and verification of prediction model

As shown in Fig. 5, the relationship between clinicopathological factors, including the expression level of CMTM6, and HCC patient prognosis was fully investigated, and the nomogram was drawn by R software, so as to more intuitively understand the predictive ability of each clinicopathological factor for the prognosis of HCC patients. Different clinicopathological factors were assigned corresponding scores, and the total score was obtained by summation. Finally, each HCC patient's 1-year and 3-year survival rates following surgical treatment were assessed based on the overall score. The higher the total score on the nomogram, the worse the prognosis.

The results showed that the cohort's prediction model had a C-index of 0.822 and that the prediction model's area under the ROC curve was 0.77 for the 1-year survival rate and 0.69 for the 3-year survival rate, as shown in Fig. 6. Due to the small sample size selected in this study, Bootstrap was used for internal verification of the model, and the calculated result was 0.65 (Accuracy>0.5), indicating good stability of the model.

The net income and forecasting ability of the nomogram

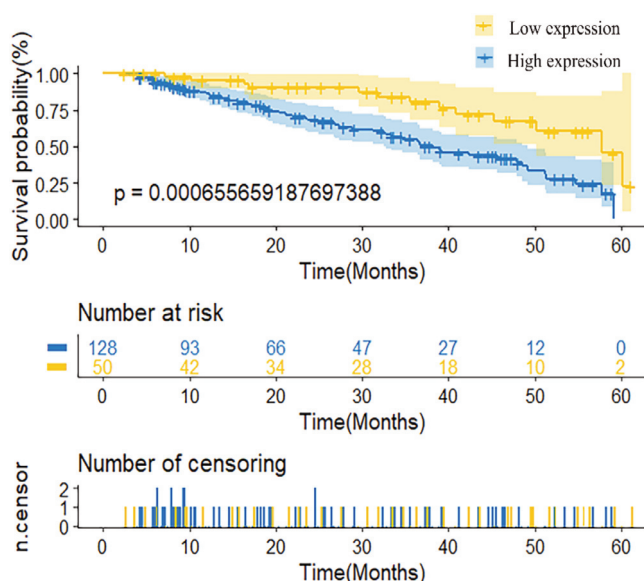The decision curve analysis and calibration curve, respectively, were used to describe the net income and

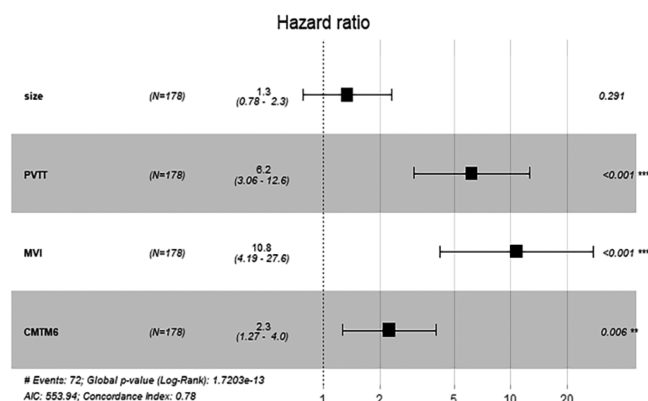**Fig. 3.** Effect of CMTM6 expression on the survival of HCC patients.

Table 2. Univariate and multivariate analysis of the correlation between clinicopathological parameters and prognostic significance of HCC patients.

| Clinicopathological feature	Univariate		Multivariate
	HR (95%CI)	P	HR (95%CI)
Age	0.99(0.61-1.6)	0.963	
Sex	0.98(0.58-1.65)	0.934	
Tumor size	1.78(1.11-2.86)	0.017**	1.34(0.28-1.06)
Stage of T	0.88(0.68-1.13)	0.325	
Distant metastasis	0.81(0.5-1.32)	0.400	
LNM	0.71(0.4-1.26)	0.239	
TNM Stage	1.07(0.82-1.4)	0.594	
HBsAg	0.83(0.52-1.32)	0.428	
AFP	0.83(0.52-1.33)	0.436	
PVTT	1.94(1.2-3.13)	0.007**	6.22(3.06-12.6)
CMTM6	2.13(1.29-3.52)	0.003**	2.23(1.27-4.03)
MVI	5.29(2.52-11.13)	<0.001**	10.76(4.19-27.6)

Fig. 4. The HR and 95% CI of PVTT, MVI and CMTM6 for OS.
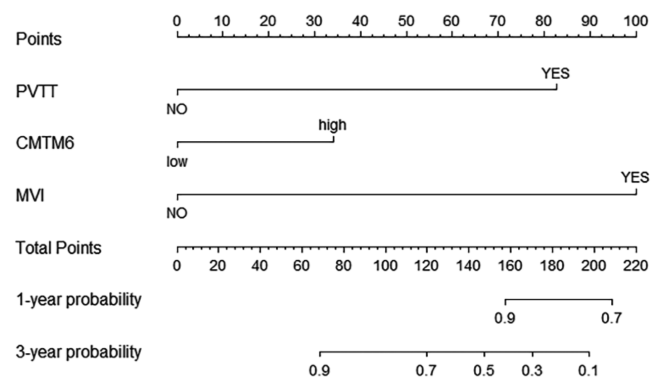
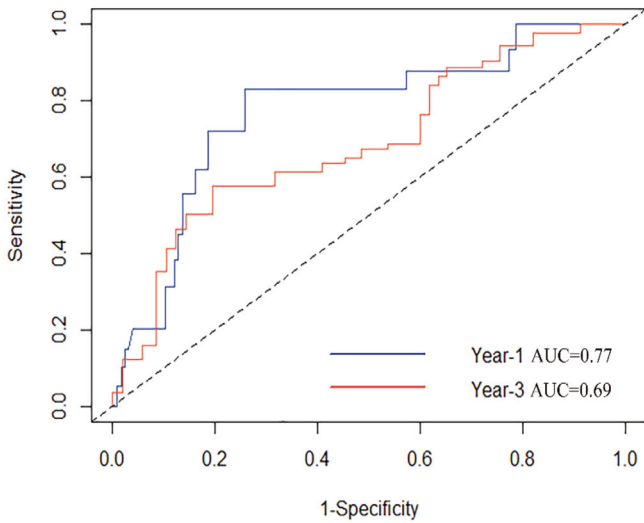**Fig. 5.** Nomogram of CMTM6 for predicting OS after curative resection of HCC.

Fig. 6. ROC curve of 1-year and 3-year survival rate of prognostic nomogram.
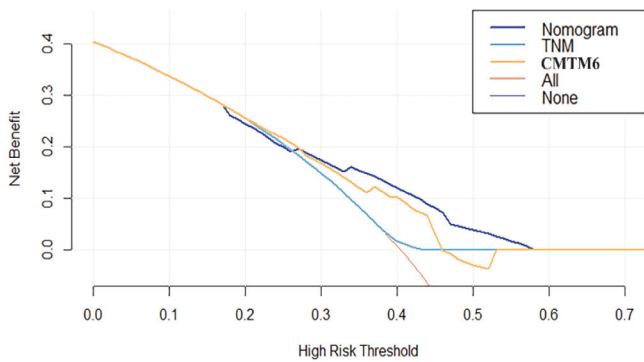

Fig. 7. The DCA curve of CMTM6, TNM system and Nomogram.

forecasting capability of the nomogram. The capacity of the nomogram to evaluate patient outcomes throughout the majority of plausible threshold probability ranges is illustrated in Figure 7 by the decision curve analysis of OS, which demonstrates that the nomogram has a larger overall net benefit than the conventional TNM approach. The expression level of CMTM6 also has higher clinical significance. The net benefit value of CMTM6 and its nomogram is larger than other assessment indices, particularly when the threshold is 0.3-0.5. The proximity between the risk predicted by the nomogram and the actual risk can also be compared using the calibration curve. Figure 8 displays the precise nomogram calibration for the 1-year and 3-year OS forecasts. The aforementioned outcomes show that the nomogram we created is more effective at predicting the survival outcome of HCC patients.

Discussion

HCC has ahidden onset, frequent metastasis and high postoperative recurrence rate, and its prevalence rate has been on the rise in recent years (Maluccio and Covey, 2012). Harnessing the immune system to fight malignancies has become the focus of cancer treatment after traditional surgery, chemotherapy, ablative therapy and radiation. Blocking immune checkpoints (such as PD-L1) is one of the most promising ways to activate therapeutic antitumor immunity. Since PD-L1 on the surface of tumor cells can inhibit the activity of T cells, thus achieving the immune escape of tumor cells, downregulating the expression of PD-L1 can reduce the inhibitory effect of PD-1/PD-L1 axis on T cells, block immune checkpoints, and achieve an anti-tumor effect (Qin et al., 2019). Studies have found (Burr et al., 2017; Yaseen et al., 2022) that the PD-1/PD-L1 pathway is closely related to the occurrence, development and
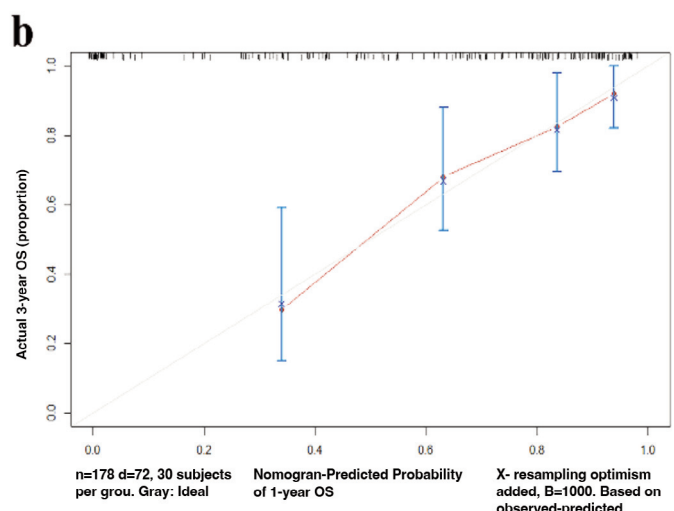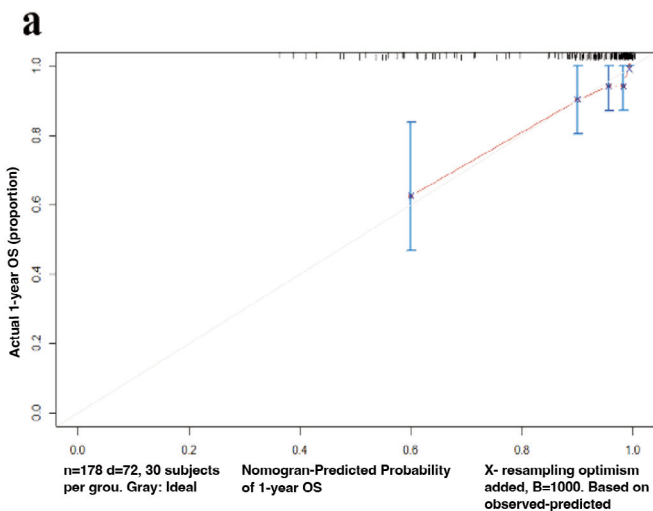

Fig. 8. Calibration curves for 1-year **(a)** and 3-year OS **(b)** of nomogram predictions.

prognosis of HCC, which opens a new situation for the immunotherapy of HCC.

Two articles published in Nature in 2017 simultaneously reported that CMTM6 combined with PD-L1 could stabilize the expression of PD-L1 in the plasma membrane, thus preventing PD-L1 from being degraded by lysosomes (Burr et al., 2017; Yaseen et al., 2022). The results indicate that CMTM6 is a major positive regulator of PD-L1 expression at the protein level. Previous studies have found that high expression of CMTM6 in some malignant tumors is associated with poor prognosis of patients. Li et al. (2020b) first reported that CMTM6 is positively correlated with the expression of PD-L1 in gastric cancer tissues, and high expression level of CMTM6 in patients with gastric cancer is associated with shorter overall survival. Subsequently, Zhang et al. (2021) also confirmed the research results consistent with Schmid et al. (2018). In addition, Li et al. (2020a) reported that with the increase of CMTM6 and PD-L1 expression levels, the malignancy of gastric cancer also increased. According to their results, CMTM6 can be considered as a positive regulator of PD-L1 expression in gastric cancer, and the high expression of the combination of CMTM6 and PD-L1 can be used as a prognostic marker for gastric cancer. Mamessier et al. (2018) aimed to explore the prognostic value of CMTM6 expression alone or combined with PD-L1 in the gene expression database of 453 pancreatic cancer tissues. Results showed that CMTM6 increased prognostic information by stabilizing the expression of PD-L1, suggesting a synergistic effect of CMTM6 and PD-L1 in disease progression. The higher the expression of CMTM6, the shorter the overall survival of patients. Burr et al. (2017) revealed that CMTM6 is a major regulator of PD-L1 expression in melanoma, breast cancer and lung cancer cell lines. Mezzadra et al. (2017) showed that inhibition of CMTM6 expression would lead to impaired expression of PD-L1 in all human tumor types. Therefore, CMTM6 can effectively inhibit T cell activity by stabilizing PD-L1 on the surface of tumor cells, so inhibition of CMTM6 may increase the effect of blocking PD-L1 pathway.

It is worth noting that CMTM6 also plays an important role in the tumor immune microenvironment (TIME). The immune microenvironment is closely related to the expression of PD-L1 in HCC tissue. Research has found an association between PD-L1 and TIME in HCC patients. Yugawa et al. (2021) identified a significant correlation between PD-Ll and CD68+ cells, and found that CMTM6 is also related to CD68+ cells in HCC. These results indicate that TIME may affect the expression of PD-L1, and maintain tumor immunity through the expression of CMTM6 in the HCC immune microenvironment, promote disease progression, and lead to poor prognosis of patients. In addition, different research groups have confirmed that CMTM6 can be directly or indirectly involved in regulating the expression of PD-L1 in different immune cells (including dendritic cells, macrophages and monocytes)

(Yaseen et al., 2022). Shang et al. (2020) verified the association between the protein CMTM6 and PD-L1 levels in lung cancer specimens using IHC, and found that CMTM6 and PD-L1 were positively correlated in both mRNA and protein levels. In addition, they detected differentially altered genes in CMTM6 expression levels between the high and low groups. In lung cancer patients, B cell memory, T cell memory tests, T cell follicular helper cells, macrophages M0, macrophages M1, and neutrophils were significantly different between the high and low CMTM6 expression groups. It was verified that CMTM6 was closely related to the infiltration of immune cells in the microenvironment. It is precisely the stable regulation of PD-L1 expression by CMTM6 and its role in the tumor immune microenvironment that makes CMTM6 play an important role in T cell activation and anti-tumor response, and become a potential target for tumor immunotherapy. However, more large-scale clinical studies are still needed to confirm the role of CMTM6 in different cancer types and other non-cancerous diseases.

Regarding the expression of CMTM6 in HCC, several recent studies have investigated the role of CMTM6 in HCC, but the results have been inconsistent. Zhu et al. (2019) compared the expression of CMTM6 in 75 pairs of HCC and adjacent non-tumor tissues by IHC, and they found that CMTM6 was at a low expression level in HCC and was associated with tumor metastasis and low survival rate of patients. Liu et al. (2021) showed that the expression of CMTM6 was up-regulated, especially in the MTM subtype of Liang-giant hepatocellular carcinoma. Muranushi et al. (2021) evaluated CMTM6 expression in 84 HCC samples by IHC, and the CMTM6 membrane expression rate was higher in cancer tissues (25%) than in adjacent tissues (7%). A number of studies have found that the protein level of CMTM6 in HCC is higher than that in non-tumor tissues, which may be partly due to the small sample size of Zhu's research team (Zhu et al., 2019). The IHC detection in this study showed that the expression of CMTM6 gene in HCC tissues was significantly higher than that in nearby tissues, and its expression was significantly correlated with T staging, distant metastasis, PVTT, and AFP. This result was supported by the fact that the expression of PD-L1 in HCC tissues was higher than that in adjacent tissues and was significantly correlated with a number of clinicopathological features (Yugawa et al., 2021). This study analyzed the survival prognosis of patients with high and low expression of CMTM6, and the results showed that the overall survival time of patients with high expression of CMTM6 was shorter than that of patients with low expression. COX regression analysis confirmed that PVTT (p<0.001), MVI (p<0.001) and CMTM6 expression levels (p=0.006) were prognostic factors for HCC patients. One of the most frequent side effects of HCC is PVTT (Wang et al., 2019). HCC with PVTT usually indicates poor prognosis, which has many characteristics, including rapid disease progression,

deterioration of liver function, complications related to portal hypertension, and poor tolerance to treatment (Chen et al., 2020; Qiu et al., 2021). If not treated, the median survival is 2-6 months (Soin et al., 2020). Zhang et al. (2020) found that PLC patients varied according to histological subtypes, and once PVTT occurred, the survival outcome after surgical resection was equally poor in HCC, ICC and CHC subgroups. MVI is defined as the presence of tumor cells in the vascular space lining the portal vein, large cystic vessels or endothelial cells (Du et al., 2014), which is difficult to detect before hepatectomy and can only be observed under a microscope (Huang et al., 2013). MVI has been further validated as an independent predictor of overall and disease-free survival following hepatectomy and is the most significant risk factor significantly related with early postoperative HCC recurrence among all the risk variables for HCC recurrence (Zhao et al., 2017; Cai et al., 2019; Li and Zhang, 2019). The three factors listed above are highly associated with the prognosis of HCC and have significant clinical value.

A variety of prognostic charts based on demographic and clinicopathological parameters such as age, sex, ethnicity, tumor site, and depth of tumor invasion have been developed to predict survival in patients with HCC, but individual heterogeneity is ignored by treating people at the same stage with similar approaches. High expression of CMTM6 is closely associated with the occurrence and development of multiple cancers and poor prognosis, but it is not used in prognosis assessment. In order to comprehensively improve prognostic accuracy and develop a multi-parameter prognostic model, this study creatively established a clinical prognostic model for liver cancer patients based on the expression of CMTM6, aiming at comprehensively improving the prognostic accuracy for patients. Compared with other Nomograms, it can fully reflect the prognosis of the body and is economical, convenient and accurate. We used PVTT, MVI, and CMTM6 determined by Cox proportional hazard regression analysis as independent prognostic factors for OS, and established a histogram containing these three prognostic factors to predict OS in HCC patients and conducted internal verification. Compared with the traditional TNM stage system, the nomogram has higher prediction accuracy and discrimination capacity. The actual value and anticipated value are remarkably consistent, as shown by the correction curve, which guarantees the nomogram's dependability when used repeatedly. The area analysis under the ROC curve shows the superiority of the line graph. For evaluating clinical effectiveness, DCA is an accurate and effective method, which achieves the maximum net benefit and is better than the ROC curve (Rousson and Zumbrunn, 2011). DCA can be used to evaluate and compare many predictive models considering clinical efficacy in combination with clinical outcomes (Kerr et al., 2016; Lamain-de Ruiter et al., 2016). Additionally, the DCA curve can demonstrate the higher utility of the nomogram by more accurately

reflecting its clinical application value than the TNM method.

Shortcomings of this study: (a) The selected research objects are from the same center, so a larger sample size and multiple center studies should be considered; (b) No validation cohort was set for external validation of the model; (c) Few clinical indicators were selected and most of them were related to pathological findings. The above limitations may affect the accuracy of the prediction model to a certain extent. The prediction model needs to be further improved and verified in other centers or multicenter cohorts to further improve the accuracy and applicability of the model.

To sum up, this study combined immuno-histochemical validation with a clinical prediction model, found that CMTM6 was highly expressed in HCC tissue, and suggested that the prognosis of HCC patients was poor. The expression level of PVTT, MVI, CMTM6 was an independent risk factor affecting the prognosis of HCC patients, and suggested that CMTM6 was a potential molecular marker for predicting the prognosis of HCC patients. The 1-year and 3-year survival rates of HCC patients after resection can be precisely predicted by the survival prediction model developed based on the aforementioned criteria. The results show that the nomogram has the best prediction ability, and its stability is confirmed among the groups. In future, the prognostic risk factors and applicability of HCC patients should be further expanded, and the prediction model should be enriched, so as to apply the prediction model to clinical practice and guide individualized treatment. In addition, considering the limitations of the study, our work did not show the possible mechanism of CMTM6 associated with poor prognosis of HCC. Therefore, further work is needed.

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