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Nucleolin as a Potent Biomarker for Predicting Tumor Recurrence among Patients with Hepatocellular Carcinoma after Transplantation

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ABSTRACT

Background & Aims: Tumor recurrence poses a significant challenge post-liver transplantation (LT) for hepatocellular carcinoma (HCC), necessitating the development of more precise predictive tools. In this study we aimed to investigate nucleolin as a biomarker for predicting HCC recurrence after LT.

Methods: A cohort of 241 HCC patients undergoing LT was enrolled from three medical facilities spanning January 1, 2015, to December 31, 2017. Utilizing tissue microarrays, we assessed the predictive potential of nucleolin. Survival analyses, including Kaplan-Meier and log-rank tests, were employed to scrutinize overall survival and recurrence-free survival. Based on univariate and multivariate Cox regression analyses of preoperative parameters, nomogram and risk score were designed to predict HCC recurrence and determine the effectiveness of the model.

Results: The expression of nucleolin in HCC nucleus was increased. High nucleolin expression in tumor tissues correlated with poor overall survival and recurrence-free survival (5-year overall survival ratios: 34% vs. 64.8%, 5-year recurrence-free survival ratios: 36.1% vs.67.9%, all p<0.001). Multivariate *Cox* regression analysis identified nucleolin expression score, Hangzhou criteria, HBsAg, tumor differentiation and alpha-fetoprotein level as independent risk factors for tumor recurrence in HCC patients post- LT. A new nomogram is established based on the above risk factors with effective prediction efficiency (area under time-dependent receiver operating characteristic =0.742, concordance-index =0.7742).

Conclusions: Nucleolin can be combined with a nomogram as an effective tool to predict recurrence in HCC patients following LT.

Key words: hepatocellular carcinoma – tumor recurrence – nucleolin – liver transplantation - nomogram

Abbreviations: AFP: alpha-fetoprotein; AUROC: area under the receiver operating characteristic curve; HBsAg: hepatitis B antigen; HCC: hepatocellular carcinoma; HRTR: high risk of tumor recurrence; IHC: immunohistochemistry; LRTR: low risk of tumor recurrence; LT: liver transplantation; MVI: microvascular invasion; NCL: nucleolin; OS: overall survival; RFS: recurrence-free survival; ROC: receiver operating characteristics; RR: relative risk; TACE: transarterial chemoembolization; TMA: tissue microarray.

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as the fifth most prevalent cancer globally and stands as the third leading cause of cancer-related mortality [1, 2]. In China, HCC is the second leading cause of cancer-related death among all cancers with 14% of deaths [3]. Liver transplantation (LT) is considered the most viable approach for the comprehensive treatment of HCC, significantly enhancing patient survival rates [4-6]. However, patients with post-LT are still at risk for the recurrence of HCC, especially those in the terminal stage [7, 8]. The recurrence rate of HCC after LT is as high as 10-15% [9, 10]. Patients experiencing HCC recurrence face a bleak prognosis, with reported median survival rates as low as 8.7 months. Hence, it is crucial to promptly assess the risk factors for postoperative recurrence in LT patients and implement appropriate interventions to enhance their prognosis.

The early predictors after transplantation mainly included clinical markers, tumor morphological information and pathological features [11, 12]. Jung et al. [13] discovered, through pre-operative imaging techniques, that HCC tumors with a maximum diameter > 3 cm independently correlated

with recurrence within one year following surgery. Besides, microvascular invasion (MVI) in histological studies is also an adverse factor leading to postoperative recurrence [14]. More notably, biomarkers play an indispensable role in assessing prognosis and predicting tumor recurrence. Alpha-fetoprotein (AFP), a glycoprotein that is often elevated in HCC patients, is an effective predictor of early HCC recurrence [15]. Nevertheless, the method of assessing the risk of recurrence solely by the serum AFP level of patients is incomplete. Therefore, there is a growing need for more precise and efficient methods to evaluate the risk of HCC recurrence.

Nucleolin (NCL) is a multifunctional protein mostly concentrated in the nucleus, which can participate in biological processes such as ribosome biogenesis, DNA repair and recombination, epigenetic modification, and maintenance of gene stability [16]. In the onset and progression of cancer, NCL has been linked to various types of cancers. Wei et al. [17] demonstrated that the inhibition of the NCL/ERα36 pathway can reduce cell cycle related proteins and thus inhibit the growth of breast cancer cells. Not only that, NCL has been demonstrated to promote tumor growth and blood vessel formation through multiple pathways such as Fas ligand binding, thus promoting metastasis [18, 19]. In the study on HCC, Burbano et al. [20] discovered that C20orf204, a splicing variant of Linc00176, and C20ORF204-189AA, which comprises 189 amino acids, can upregulate specific nuclear proteins and thereby promote the proliferation of HCC. Even so, clinical studies on NCL in HCC remain limited. Therefore, we chose to investigate its relationship with prognosis and tumor recurrence after liver transplantation.

In this study, we unveil NCL as a potentially significant biomarker for prognosis and tumor recurrence post-LT. We established a prognostic recurrence model of HCC based on a NCL expression score. This study provides guidance and a new direction for clinical prediction of HCC recurrence.

METHODS

Data and Resources

A total of 241 patients enrolled between January 1, 2015 and December 31, 2017 were included in the study cohort. Among the patients, 146 were from the First Affiliated Hospital of Zhejiang University School of Medicine, 78 were from Beijing Chaoyang Hospital of Capital Medical University, and 17 were from the Gulou Hospital affiliated to Nanjing University School of Medicine. All patients were randomly selected from LT patients with histopathological confirmed HCC, and all patients were fully informed and provided written informed consent prior to surgery. The study adhered to the principles of the Declaration of Helsinki. All research protocols received approval from the Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine. Human subject studies were reviewed and endorsed by the same Ethics Committee. Participants' legal guardians or next of kin provided written informed consent to participate in the study.

The follow-up ended on April 3, 2022, with a median follow-up of 3.25 years. Overall survival (OS) was computed from the date of surgery until either death or the last documented follow-up, with the patient's status at the time of last contact being recorded. Recurrence-free survival (RFS) was determined from the date of surgery to the occurrence of recurrence, death, or the last documented follow-up, and recurrences were confirmed using contrast-enhanced imaging according to standard guidelines for HCC. Table I outlines the baseline characteristics of 241 patients. The median age for all patients was 52 years, with 92.5% being male. 221 patients (91.7%) were infected with HBV and 228 patients (94.6%) had cirrhosis. A total of 102 patients (42.3%) underwent transarterial chemoembolization (TACE) before surgery, while the remaining 139 patients (57.7%) did not receive any preoperative treatment. Serum AFP levels were elevated (20 ng/mL) in 147 patients (61.0%). 141 cases (58.5%) were well-differentiated or moderately differentiated, and 100 cases (41.5%) were poorly differentiated. 108 patients (44.8%) experienced tumor recurrence after transplantation and 133 patients (55.2%) did not.

Tissue Microarray and Immunohistochemical Staining

Hepatocellular carcinoma tissue microarray (TMA) was prepared by Shanghai Outdo Biotech Company (Shanghai, China). TMA contained 241 HCC tissues. Immunohistochemistry (IHC), TMA sections were treated with anti-NCL. The immunohistochemical staining was scored independently by two pathologists who were unaware of the patients' clinical characteristics. The final immune reactivity score was determined by multiplying the intensity score (ranging from 0 to 3 for no staining, weak staining, moderate staining, and strong staining, respectively) by the percentage score (ranging from 0 to 4 for <5%, 5%-25%, 26%-50%, 51%-75%, and >75% positive cells, respectively), resulting in a composite score ranging from 0 to 12. According to the final immune response score, the immunohistochemical results were divided into 0-3 as negative (-) and 4-12 as positive (+).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (Version 9; GraphPad, La Jolla, CA, USA) and IBM SPSS Statistics (Version 26; IBM Corporation, New York, USA). Parametric tests are employed to compare continuous variables, while chi-square tests are utilized for comparing categorical variables. The Kaplan-Meier method and log-rank test were applied to analyze both OS and RFS. Additionally, multivariate proportional hazards *Cox* regression analysis was conducted to identify independent prognostic factors. The variables compared in the univariate analysis in this study included gender (male/female), age (<60 years /≥60 years), and pre-LT TACE (yes/no), hepatitis B antigen (HBsAg; positive/negative), cirrhosis (yes/no), end-stage liver disease model score, Hangzhou criteria (no major vessel invasion, meeting one of the following criteria: total tumor diameter of 8 cm, AFP and differentiation are not considered, or total tumor diameter of 8 cm, AFP 400 ng/mL, differentiation is good), and the Milan standard (5cm for a single lesion or 3 cm for each of 2 or 3 lesions) (in/over). These variables were analyzed to assess the determinants of survival after LT. Variables that showed statistical significance in the univariate analysis were included in the forward stepwise multifactor *Cox* proportional hazards analysis to evaluate the relative risk (RR) of postoperative survival.

Nomogram and calibration plots were established using R studio based on independent prognostic factors. The consistency index (C-index) and area under the receiver operating characteristic curve (AUROC) over time were employed to assess the discrimination ability. Calibration diagram was utilized to evaluate the calibration capability. The C-index and AUROC values fall within the range of 0.5 to 1.0, with 0.5 indicating a random probability and 1.0 representing a perfect fit. Typically, C-index and AUROC values exceeding 0.7 suggest a reasonable estimate. A p value ≤0.05 was considered to be statistically significant.

RESULTS

Immunohistochemistry data showed that NCL was significantly more distributed in the nucleus of tumor cells than in the cytoplasm (Fig. 1A). Based on the difference in the expression of NCL in tumors, patients were divided into NCL^{high} (n=125) and NC^{low} (n=116) groups. Between the two groups, patients in the NCL^{high} group showed significantly lower OS and RFS than those in the NCL^{low} group (Fig. 1B-C). The 1-year, 3-year and 5-year OS ratios of NCL^{high} and NCL^{low} groups were 56.4%, 42.5% and 34.0% vs. 78.8%, 69.7% and 64.8%, respectively (p<0.05, Figure 1B). The 1-year, 3-year, and 5-year RFS ratios in the NCL^{high} and NCL^{low} groups were, 45.0%, 37.5% and 36.1% vs. 69.0%, 67.9% and 67.9%, respectively (p<0.05, Fig. 1C).

Besides, high NCL expression in HCC demonstrated a significant association with male patients (p=0.033), preoperative TACE (p=0.018), greater maximal tumor diameter (p=0.015), greater total tumor diameter (p=0.028), and exceedance of the Milan criterion (p=0.013, Table I). These findings suggest that HCC patients exhibiting high NCL expression face an elevated risk of death and tumor recurrence following LT.

To further analyze the association between NCL expression and compliance with the Milan and Hangzhou criteria for post-LT OS and RFS rates in HCC patients, we conducted further grouping and comparison. According to the Milan criteria, HCC patients were divided into conforming Milan criteria (n=73) and exceeded the Milan criteria (n=168, Table II). Fig. 2A-B shows a comparison of OS and RFS for groups that conforming Milan standard with NCL^{high}, exceed the Milan criteria with NCL^{high}, conforming Milan criteria with NCL^{low} and exceed the Milan criteria with NCL^{low}. Compared with patients conforming Milan criteria, the NCL^{high} group showed a significantly lower OS and RFS ratios than the NCL^{low} group (p=0.0145, p=0.0118). In the subset of patients exceeding the Milan criteria, the NCL^{high} group also had lower OS and RFS rates than the NCL^{low} group (p=0.0088, p=0.0041). Similarly, in the NCL^{high} group, patients exceeding the Milan criteria exhibited lower survival rates and higher recurrence rates after LT compared to those conforming the Milan criteria (p=0.0088, p=0.0003). Patients exceeding the Milan criteria demonstrated lower survival rates and higher recurrence rates compared to those conforming the Milan criteria in the NCL^{low} group (p=0.0011, p<0.0001).

Figure 2C-D shows a comparison of OS and RFS for groups that conforming Hangzhou standard with NCL^{high}, exceed the Hangzhou criteria with NCL^{high}, conforming Hangzhou criteria with NCL^{low} and exceed the Hangzhou criteria with NCL^{low}.

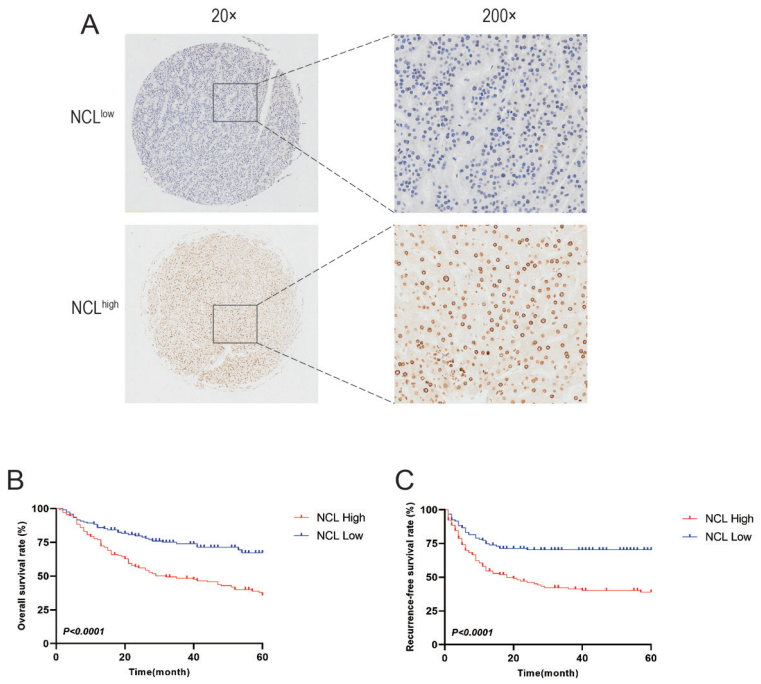

Fig. 1. The relationship between NCL and prognosis after liver transplantation in HCC patients. (A) Representative images of NCL^{low} and NCL^{high} expression in HCC tissues detected by IHC. (B) Kaplan-Meier survival curves show OS(P<0.05) in patients with NCL^{low} (n=116) and NCL^{high} (n=125). (C) Kaplan-Meier survival curves show RFS(p<0.05) in patients with NCL^{low} (n=116) and NCL^{high} (n=125). NCL: nucleolin; HCC: hepatocellular carcinoma; IHC: immunohistochemistry; OS: overall survival; RFS: recurrence free survival.*p<0.05.

Table I. Baseline characteristics of 241 HCC patients undergoing liver transplantation

Characteristics	n
Age (year) [median (IQR)]	52 (14)
Gender	
Male	223
Female	18
Liver cirrhosis	
No	13
Yes	228
Preoperative TACE	
Not received	139
Received	102
Preoperative AFP level (ng/mL) [median (IQR)]	52.50 (1219.25)
HBsAg	
Negative	30
Positive	211
PVTT	
without PVTT	192
with PVTT	49
MELD score	
<25	193
≥25	48
Child-Pugh grade	
A	82
B	85
C	74
Tumor number	
<3	144
≥3	97
Maximal tumor diameter(cm)	
≤5	147
>5	94
Total tumor diameter(cm)	
≤5	77
>5	164
Tumor differentiation	
Well/moderate	141
Poor	100
Post-transplant recurrence	
No	133
Yes	108
Hangzhou criteria	
Within	139
Beyond	102
Milan criteria	
Within	73
Beyond	168

AFP: alpha fetoprotein; TACE: transarterial chemoembolization; PVTT: portal vein thrombosis; IQR: interquartile range; HBs Ag: hepatitis B antigen.

Table II. Clinicopathological characteristics of patients with HCC based on expression of NCL

Characteristics	NCL^{low} group (n=116)	NCL^{high} group (n=125)	p
Age(year)			0.438
<60	88	100	
≥60	28	25	
Gender			0.033
Male	103	120	
Female	13	5	
Cirrhosis			0.320
No	8	5	
Yes	108	120	
Preoperative TACE			0.018
No	76	63	
Yes	40	62	
AFP (ng/mL)			0.128
≤20	51	43	
>20	65	82	
HBsAg			0.542
Negative	16	14	
Positive	100	111	
PVTT			0.408
without PVTT	95	97	
with PVTT	21	28	
MELD score			0.973
<25	93	100	
≥25	23	25	
Child-Pugh grade			0.160
A	33	49	
B	42	43	
C	41	33	
Tumor number			0.332
<3	73	71	
≥3	43	54	
Maximal tumor diameter(cm)			0.015
≤5	80	67	
>5	36	58	
Total tumor diameter (cm)			0.028
≤5	45	32	
>5	71	93	
Tumor differentiation			0.109
well/moderate	74	67	
poor	42	58	
Hangzhou criteria			0.775
within	68	71	
beyond	48	54	
Milan criteria			0.013
within	44	29	
beyond	72	96	

For abbreviations see Table I.

Compared with patients conforming Hangzhou criteria, the NCL^{high} group showed a significantly lower OS and RFS ratios than the NCL^{low} group (p=0.0003, p<0.0001).

The OS and RFS rates in the NCL^{high} group were also lower than the NCL^{low} group (p=0.0169, p=0.0198) in the subset of patients exceeding the Hangzhou criteria. In addition, patients in the NCL^{high} group exceeding the Hangzhou criteria had significantly higher mortality and recurrence rates than the conforming Hangzhou criteria group (both p<0.0001). Patients exceeding the Hangzhou criteria demonstrated lower survival rates and higher recurrence rates than those conforming the Hangzhou criteria in the NCL^{low} group (both p<0.0001). All the aforementioned data collectively indicate that HCC patients with high NCL expression exhibit lower OS and RFS, irrespective of whether patients are stratified based on the Milan criteria or the Hangzhou criteria.

To further assess the potential risk factors for tumor recurrence in HCC patients after LT, univariate and multivariate *Cox* regression analyses were conducted for the clinical features listed in Table III.

Table III shows the univariate *Cox* regression of HCC recurrence, including NCL expression score, AFP level, tumor differentiation, Hangzhou criteria (all p<0.001), preoperative TACE (p=0.002) and HBsAg (p=0.015), all of which are significantly associated with the RFS of HCC patients after LT. Multivariate regression analysis revealed that NCL expression score, Hangzhou criteria (p<0.001), HBsAg (p=0.030), tumor differentiation (p=0.048) and AFP level (p=0.019) are independent risk factors for tumor recurrence. Based on these six factors, we developed a novel nomogram to predict tumor recurrence within 5 years following LT in HCC patients (Fig. 3A). The model demonstrated accuracy, with a C-index of 0.742 [95% confidence interval (CI): 0.701- 0.783]. Calibration plots confirmed that the nomogram was in good agreement with 5-year tumor recurrence survival predictions (Fig. 3B). In addition, time-dependent receiver operating characteristics (ROC) were employed to assess the prognostic sensitivity and specificity of the model. AUROC provided a summary of the ROC results. Compared to the Milan criteria, the AUROC of the whole set was 0.7742, which was better than the Milan standard AUROC (AUC=0.6425, Figure 3C). To sum up, our newly established model for predicting HCC recurrence has better discrimination and calibration capabilities.

In addition, we constructed a novel risk score that can effectively predict OS and RFS. This risk score was calculated using the following formula: risk score=0.422×AFP level (0, ≤20 ng/ml; 1, >20 ng/ml) + 0.855×NCL expression (0, <4; 1, ≥4) + 0.205×HBsAg (0, positive; 1, negative) + 0.592×tumor

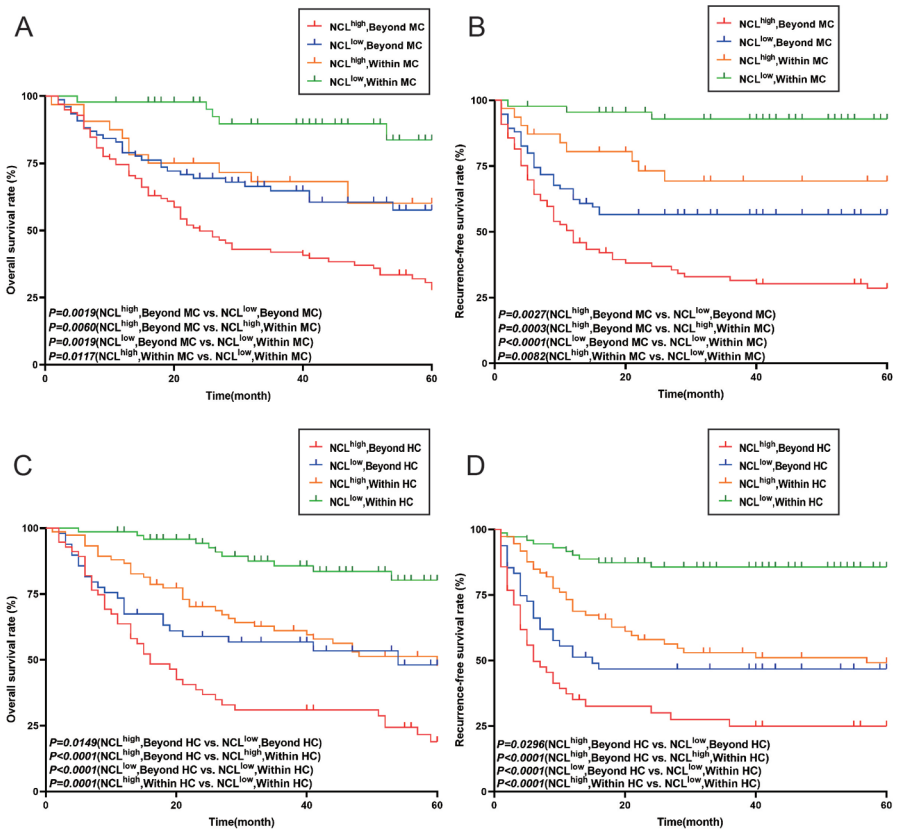

Fig. 2. Survival analysis of subgroup studies with NCL expression and Milan or Hangzhou criteria. (A-B) Kaplan-Meier survival curves representing OS (A) and RFS (B) in HCC patients with NCL^{low} & within MC (n=44), NCL^{high} & within MC (n=29), NCL^{low} & beyond MC (n=72) and NC^{Lhigh} & beyond MC (n=96); (C-D) Kaplan-Meier survival curves representing OS (A) and RFS (B) in HCC patients with NCL^{low} & within HC (n=68), NCL^{high} & within HC (n=71), NCL^{low} & beyond HC (n=48) and NCL^{high} & beyond HC (n=54). NCL, nucleolin; HCC, hepatocellular carcinoma; OS: overall survival; RFS, recurrence free survival; MC: Milan criteria; HC: Hangzhou criteria; *, p<0.05; **, p<0.01.

Table III. Univariable and multivariable Cox-regression analyses on risk factors of recurrence-free survival in 241 HCC patients undergoing liver transplantation

Characteristics	Comparison	Univariate Analysis			Multivariate Analysis		
		HR	95%CI	p	HR	95%CI	p
NCL expression score	<4 vs. ≥4	2.321	1.554-3.468	**<0.001**	2.088	1.389-3.138	**<0.001**
Age	<60 years vs. ≥60 years	0.643	0.387-1.067	0.087			
Sex	male vs. female	0.595	0.242-1.460	0.257			
Preoperative TACE	yes vs. no	1.823	1.248-2.661	**0.002**	1.455	0.989-2.142	0.057
AFP level	≤20 ng/ml vs. >20 ng/ml	2.245	1.466-3.438	**<0.001**	1.682	1.088-2.601	**0.019**
MELD score	≤25 vs. >25	1.135	0.711-1.814	0.595			
Child-Pugh grade	A vs. B	1.273	0.791-2.049	0.946			
	A vs. C	1.160	0.715-1.882	0.548			
HBsAg	positive vs. negative	2.777	1.219-6.330	**0.015**	2.272	0.989-5.219	**0.030**
Cirrhosis	yes vs. no	1.023	0.449-2.330	0.958			
Tumor differentiation	well/moderate vs. poor	2.788	1.898-4.095	**<0.001**	1.526	1.001-2.325	**0.048**
Hangzhou criteria	within vs. beyond	2.980	2.024-4.387	**<0.001**	2.278	1.503-3.453	**<0.001**

For abbreviations see Table I. NCL: nucleotin.

differentiation (1, well/moderate; 2, poor) + 0.824×Hangzhou criteria (0, within; 1, beyond). According to the Youden Index, the optimal cut-off value for the risk score was 1.775.

The whole cohort was re-divided into a low risk of a tumor recurrence group (LRTR, risk score ≤1.775, n=134) and high risk of a tumor recurrence group (HRTR, risk score >1.775, n=107).

Kaplan-Meier analysis indicated that HRTR patients exhibited a significantly higher risk of tumor recurrence compared to LRTR patients (p<0.001, Fig. 3D). Fig. 3E showed that HRTR has significantly lower overall survival compared to LRTR (p<0.001). The 1-year, 3-year and 5-year OS of LRTR and HRTR were 91.3%, 75.7% and 67.1% vs. 71.2%, 48.3% and

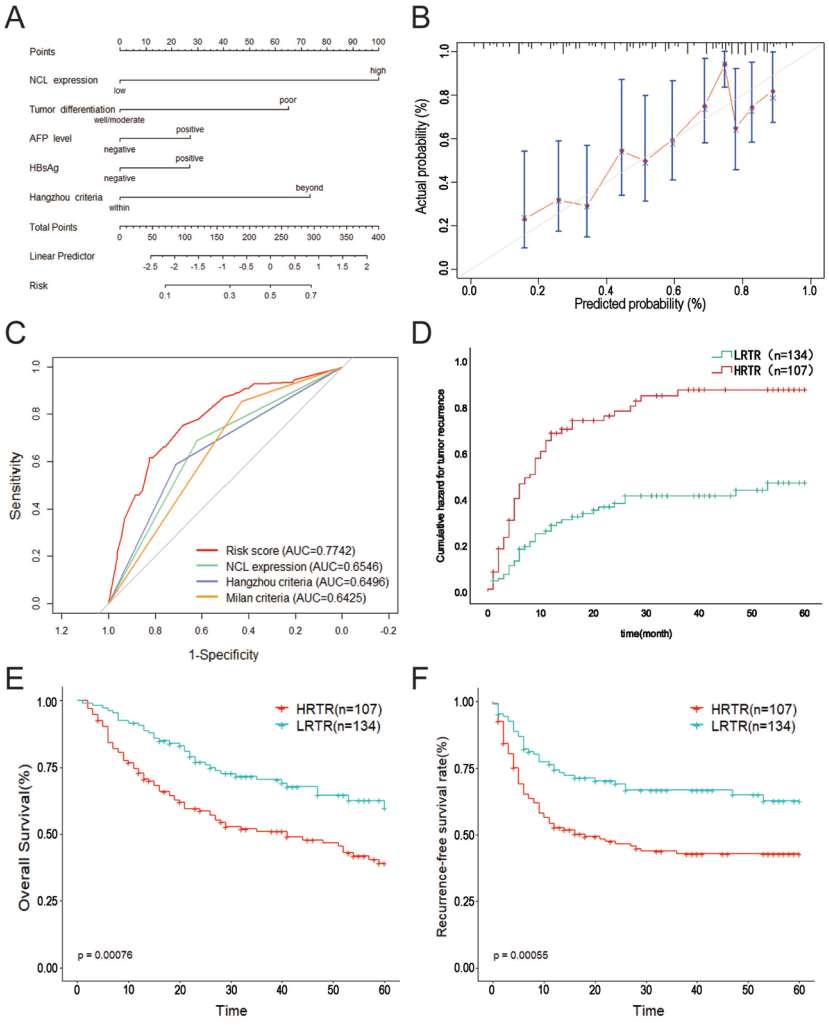

Fig. 3. Nomogram to predict HCC recurrence after transplantation and its efficacy validation. (A) Nomogram to predict 5-year recurrence-free survival of HCC patients after liver transplantation; (B) Calibration curve of the nomogram; (C) The ROC curves of Milan criteria (AUC=0.6425), Hangzhou criteria (AUC=0.6496), NCL expression level (AUC=0.6546) and risk score (AUC=0.7742) were compared to predict the prognosis of tumor recurrence in HCC patients after transplantation; (D) Kaplan-Meier survival analysis of the LRTR group and HRTR to predict the cumulative risk of recurrence after liver transplantation (p<0.001); (E-F) Kaplan-Meier survival analysis of the LRTR group and HRTR to predict OS (p<0.001) and RFS (p<0.001) afte.r liver transplantation. HCC, hepatocellular carcinoma; ROC, receiver operating characteristics; AUC, area under the curve; HRTR, high risk of tumor recurrence group; LRTR, low risk of tumor recurrence; OS, overall survival; RFS, recurrence free. **, p<0.01. ***, p<0.001

37.7%, respectively. Fig. 3F shows that the RFS of HRTR is lower than that of LRTR. Among them, the 1-year, 3-year and 5-year RFS of LRTR and HRTR were 75.3%, 64.6% and 62.6% vs. 50.4%, 41.8% and 41.8%, respectively. In summary, patients classified as LRTR based on our risk score demonstrated higher survival rates and lower recurrence rates compared to those classified as HRTR.

DISCUSSION

Recurrence after LT stands as a significant postoperative complication for HCC patients and represents one of the primary risk factors influencing patient survival. Hepatocellular carcinoma is characterized by a high recurrence rate and low survival after transplantation [21]. Therefore, early prediction of tumor recurrence is essential to improve the survival rate of HCC patients after LT. However, there is currently a lack of systematic surveillance for HCC tumor recurrence. Therefore, more accurate and effective prediction methods for HCC recurrence are required.

Studies have long established a link between tumor development and molecular biology [22]. NCL has been shown to be involved in the progression and treatment decisions of lung, breast and other malignancies [17, 23]. In HCC, Qian et al. [24] conducted proteomic analysis of HCCLM9 at an early stage and found that high expression of NCL would enhance the aggressiveness of tumors, demonstrating the research potential of NCL. Moreover, Liang et al. [25] used NCL as a targeted protein for drug delivery and constructed a functionalized AS1411 aptamer micelle for simultaneous co-delivery of adriamycin and miR-519c to effectively solve the multidrug resistance problem of HepG2 liver cancer cells. The above existing studies can prove the correlation between NCL and the function of HCC tumor cells. Our research indicates that increased levels of NCL act as a separate predictor for a reduced chance of post-surgical survival and an increased risk of tumor recurrence in HCC patients.

The Milan criteria represent the gold standard worldwide for screening HCC patients eligible for LT. On the one hand, our analysis results demonstrate that high NCL expression in HCC patients meeting the Milan criteria can be an effective indicator for predicting high mortality and recurrence rates after transplantation. On the other hand, additional survival analysis of patients exceeding the Milan criteria revealed that high expression of NCL also emerged as a potential prognostic factor for HCC patients post LT. Similarly, NCL expression was effective in grouping patients based on Hangzhou criteria for predicting survival and recurrence after LT. Alpha fetoprotein is a significant biomarker for predicting both prognosis and tumor recurrence after transplantation [15]. Recent studies indicate that AFP levels, total tumor volume, and other biomarkers such as the PIVKA score can effectively predict the survival and prognosis of patients following transplantation [26]. Nevertheless, in HCC patients with negative serum AFP, negative AFP may, to a certain extent, affect transplant evaluation and postoperative prediction in these patients. Alpha fetoprotein bound to Lens culinaris agglutinin (AFP-L3) and DES-gama-carboxyprothrombin (DCP) have been shown to jointly predict early post-transplant HCC recurrence in prospective studies [27] . In our study, we demonstrated by multivariate *Cox* regression analysis that AFP>20ng/ml was an independent risk factor for tumor recurrence.

In relation to HCC recurrence after LT, our study also found that beyond Hangzhou criteria, HBsAg positive, tumor poor differentiation, AFP level >20ng/ml, and no preoperative TACE were an independent risk factor for high recurrence rate of tumor. On this basis, we established a new nomogram containing the above independent risk factors for predicting tumor recurrence after transplantation. We also proved the accuracy of nomogram prediction. Also, we constructed risk scores based on the predicted nomogram and regrouped HCC patients into LRTR and HRTR groups. Survival analysis showed that HCC patients with HRTR had lower post-transplant survival and higher recurrence rates than HCC patients with LRTR. The above proved the accuracy of our prediction model. However, this predictive risk model needs to be confirmed with more clinical samples and is expected to provide a new method for predicting post-LT relapse in HCC patients.

This study pioneers the systematic investigation of NCL as a biomarker for predicting tumor recurrence in HCC patients post-LT. The development of a prognostic model based on NCL expression offers a novel clinical tool for HCC recurrence prediction. However, there are some limitations to this study. the sample size is relatively small and lacks multi-center representation. Future studies with larger sample sizes are needed to further verify the effectiveness of NCL as a biomarker. Additionally, while internally cross-validated, the model lacks independent external validation, which is crucial for confirming its accuracy and generalizability. Despite these limitations, this study reveals the important role of NCL in HCC recurrence and offers valuable insights for future research and clinical practice.

In summary, we reveal the ability of NCL expression in HCC patients to predict the recurrence rate after LT. Clinically, our study offers an accurate method for predicting the prognosis of HCC patients post LT, along with identifying a potential therapeutic target in NCL.

CONCLUSIONS

We demonstrated that high expression of NCL in liver tissue of HCC patients is closely associated with poor prognosis after transplantation. A nomogram model containing NCL expression score can effectively predict HCC recurrence after transplantation, and can be used as an effective tool for clinical evaluation of HCC recurrence.

Conflicts of interest: None to declare.

Authors' contributions: X.X. was involved in the conception and design of the study. R.G.C. assisted in research design and proposed key hypotheses. D.L. was responsible for data collection and collation. H.G.L. assisted with data collection and data quality control. L.B.D. managed data and build databases. X.H. managed and processed samples. Y.J. assisted in sample processing and was responsible for the experimental operations. B.C.S. participated in laboratory quality control and supervision. F.J. was responsible for statistical analysis and data processing. L.X.Y. instructed statistical analysis and data processing. G.R.C. wrote the article. Y.C.H.

interpreted and modified the results. S.S.Z. supervised the research and reviewed the manuscripts. R.L. assisted in supervising and coordinating projects.

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