Overexpression of NOP58 as a Prognostic Marker in Hepatocellular Carcinoma: A TCGA Data-Based Analysis

Jinpo Wang · Rongfeng Huang · Yuehong Huang · Yunxin Chen · Fenglin Chen

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ABSTRACT

Introduction: NOP58 ribonucleoprotein, a core component of box C/D small nucleolar ribonucleoproteins, is involved in various cell physiological processes. However, its role in hepatocellular carcinoma (HCC) remains very unclear. We aim to investigate NOP58 expression and its probable prognostic value in patients with HCC based on The Cancer Genome Atlas (TCGA) database.

Methods: RNA sequencing data and clinico-pathological characteristics of patients with HCC were collected from TCGA database. Expression of NOP58 in HCC tissues and normal tissues was analyzed by Wilcoxon rank-sum test. Patients were divided into high and low subgroups according to median expression of NOP58. Logistic regression, gene set enrichment analysis (GSEA), and single-sample gene set enrichment analysis (ssGSEA) were conducted to annotate biological function and immune infiltration of NOP58.

Results: NOP58 was significantly overexpressed in HCC tissues and correlated with significantly high tumor stage [odds ratio (OR) 10.01, 95% confidence interval (CI) 10.01–10.03; \( P = 0.003 \)], advanced pathologic stage (OR 10.02, 95% CI 10.01–10.03; \( P < 0.001 \)), advanced histologic stage (OR 10.03, 95% CI 10.02–10.04; \( P < 0.001 \)), vascular invasion (OR 10.02, 95% CI 10.01–10.03; \( P = 0.003 \)), poor performance status (OR 10.01, 95% CI 10.01–10.03; \( P = 0.003 \)), and Mut-TP53 status (OR 10.02, 95% CI 10.01–10.03; \( P < 0.001 \)). Elevated NOP58 expression had poor disease-specific survival (DSS; \( P < 0.001 \)), progression-free interval (\( P = 0.006 \)), and overall survival (OS; \( P < 0.001 \)). NOP58 expression was independently correlated with OS (HR 1.731, 95% CI 1.037–2.890; \( P = 0.036 \)). GSEA demonstrated that various cell cycle pathways along with RB-1 pathway, interleukin-10 signaling, regulation of TP53 activity, and P53 down-stream pathway were differentially enriched in NOP58 high expression phenotype. NOP58 expression was positively correlated with infiltrating the levels of T helper type 2 (Th2) cells.

Conclusions: Overexpression of NOP58 is negatively correlated with overall survival in patients with HCC and might be a potential biomarker for prognosis of HCC.
**Keywords:** Biomarker; Gene set enrichment analysis; Hepatocellular carcinoma; NOP58; Prognosis

### Key Summary Points

| The molecular mechanisms underlying hepatocellular carcinoma (HCC) metastasis remain unclear. |
|---|
| NOP58 expression might have a prognostic value in patients with HCC. |
| Various cell cycle pathways along with RB-1 pathway, interleukin-10 signaling, regulation of TP53 activity, and P53 downstream pathway were differentially enriched in NOP58 high expression phenotype. |
| NOP58 expression was positively correlated with infiltrating levels of T helper type 2 cells. |
| Overexpression of NOP58 is negatively correlated with overall survival in patients with HCC. |

### DIGITAL FEATURES

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### INTRODUCTION

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality and sixth cause of morbidity, with more than 740,000 patient deaths occurring worldwide each year [1, 2]. Although surgical resection is considered a potentially curative treatment for patients with HCC, the 5-year postoperative survival rate is 30–40% [3, 4]. The poor prognosis of patients with HCC is substantially the result of the high frequencies of tumor recurrence and occurrence of distant metastasis after curative resection [5]. In addition to surgical resection, the current treatment methods include vascular embolism, radiofrequency, radiotherapy, chemotherapy, and biotherapy [6, 7]. Even the most commonly preferred method for HCC diagnosis, serum alpha fetoprotein (AFP) monitoring is not adequate to effectively predict the postoperative survival of patients with HCC [8]. Lately, research has shown that the occurrence and poor prognosis of HCC may be related to the abnormal expression of genes [9]. With the extensive application of gene chip technologies, abundant expression profile information, and ability to screen for differentially expressed genes (DEGs), biomarkers in tumor tissues could be detected efficiently by integrating publicly available data sets [10, 11]. However, the detailed molecular mechanisms underlying the HCC metastasis remain unclear. Therefore, it is necessary to understand the molecular mechanisms for the progression of HCC, which might provide new biomarkers and novel therapeutic targets for the treatment and prognosis of HCC.

NOP58 ribonucleoprotein, also known as NOP5, HSPC120, or NOP5/NOP58, is a protein-encoding gene located on chromosome 2 [12]. The protein encoded by this gene is located primarily in the nucleolus, and its predicted molecular weight is about 59.6 kDa [12]. NOP58 comprises of a core component of box C/D small nucleolar ribonucleoproteins (box C/D snoRNPs) and serves as a pivotal component for several box C/D small nucleolar RNAs (snoRNAs), such as U3, U8, and U14 to deliver as a skeleton for the entire snoRNP complex, ultimately maintaining the cellular homeostasis [13, 14]. NOP58, NOP56, and 15.5 kDa contribute to maturation, stability, and localization of snoRNAs [15] along with playing an important role in tumorigenesis. Studies have shown that R2TP complex plays a key role in the cell physiology by influencing the level of NOP58 [16]. Moreover, NOP58 is crucial for rRNA processing and assembly [17], and alteration of ribosome composition. Dysregulation of ribosome biogenesis can serve as a marker for cancer progression and malignant transformation [17].

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Research has also demonstrated that NOP58 can improve the stability of FAM83A mRNA and promote tumor progression [18], whereas targeted repression of NOP58 can inhibit cancer cell growth as well as decrease oncogenicity [19]. Although the molecular action of NOP58 has been noticed in the aforementioned studies, whether it exerts a role in the same pattern in patients with HCC has not been well elucidated. To date, no studies have reported the clinicopathologic significance of NOP58 in patients with HCC. In this study, we used gene expression profiles and survival data obtained from The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) to investigate the differential expression of NOP58 genes in HCC. We examined the possible carcinogenic mechanism of NOP58 genes and explored the prognostic value of this gene in HCC.

All datasets were from databases of de-identified patient data and so ethics committee approval was not required.

METHODS

RNA Sequencing Data and Bioinformatics Analysis

The gene expression data (level 3 HTSeq-FPKM) for RNA sequencing and corresponding clinical information for LIHC projects were retrieved from TCGA (https://portal.gdc.cancer.gov/). The data of 371 patients with HCC were considered for further analysis, while patients without sufficient clinical information were excluded. Among them, HCC tissues of 50 patients with paired paracancerous samples were included. As there is scarce data on the relationship between expression of NOP58 gene and HCC tissue, the level 3 HTSeq-FPKM data was transformed into TPM (transcripts per million reads) and downloaded as mentioned by Vivian et al. from UCSC XENA database (https://xenabrowser.net/datapages/) [20]. Among the included patients, individual clinical information that was either unavailable or unknown was characterized as missing values.

Enrichment Analysis

A computational method termed gene set enrichment analysis (GSEA) was used to determine whether a priori defined set of genes shows statistically significant differences between two biological states (e.g., phenotypes) [21]. In this study, gene expression data were divided into a high and low NOP58 group. Based on the results of NOP58 co-expression gene analysis, the expression matrix of LIHC in HCC was constructed by TCGA. The R package clusterProfiler (version 3.14.3) [22] was used to perform GSEA between high and low NOP58 groups, and the gene set permutations were performed 1000 times for each analysis. Normalized enrichment score (NES) \( > 1 \), \( P < 0.05 \), and fast discovery rate (FDR) \( q \) value \( < 0.25 \) were considered to be significantly enriched.

Immune Infiltration Analysis

A single-sample gene set enrichment analysis (ssGSEA) was carried out using the GSVA package [23] (version 3.14.3, https://www.bioconductor.org/packages/release/bioc/html/GSVA.html) for 24 types of immune cells in tumor samples including Th1 cells, Th17 cells, Th2 cells, T cells, T helper cells, Treg, Tem, Tgd, aDC, B cells, CD8+ T cells, cytotoxic cells, DC, neutrophils, NK CD56 bright cells, NK CD56 dim cells, pDC, eosinophils, iDC, macrophages, and mast cells [24]. We quantified the relative enrichment score of every immunocyte from the gene expression profile for each tumor sample based on the published literature [24]. The degree of immune infiltration was recorded as ssGSEA enrichment score. The correlation between NOP58 and the infiltration levels of these 24 types of immunocytes was analyzed by Spearman’s rank correlation coefficient method.

Construction of Prognostic Model Based on Kaplan–Meier Survival Analysis

The prognostic model for subtype differentiation and prognosis evaluation was built on the basis of a previously published article [25].
association between the prognostic value calculated by the prognosis evaluation model and the overall survival (OS) of patients with HCC was further assessed by multivariate Cox regression analysis. The patients were divided into high and low subgroups according to the median expression of NOP58. Cox regression analyses and Kaplan–Meier curves drawn by Survminer R package (version 0.4.8) were used to compare the influence of NOP58 expression on OS, progression-free interval (PFI), and disease-specific survival (DSS) along with other clinical characteristics. The receiver operating characteristic (ROC) curve was used to analyze the efficiency in distinguishing HCC tissues from non-cancerous tissues by NOP58 gene expression level. We validated our findings with the Oncomine database using similar risk variables and clinicopathological features as reported in our study.

RESULTS
Identification of Differential Expression of NOP58 Gene in Human Tumor Tissues
To explore the role of NOP58 in determining the clinical outcomes in patients with HCC, Wilcoxon rank-sum test was used to compare the expression of NOP58 in normal samples of Genotype-Tissue Expression (GTEx) combined with TCGA and TCGA corresponding tumor samples. The results showed that NOP58 was significantly expressed in a variety of tumors (P < 0.05), including BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PAAD, READ, SARC, STAD, TGCT, THCA, THYM, UCEC, and UCS (Fig. 1), which indicates that NOP58 is closely related to cancer.

Immunohistochemical Analysis
In order to further clarify the expression of NOP58 in HCC, human liver cancer tissue chip HLivH180Su11 was used to carry out immunohistochemical experiments. A total of 85 normal and adjacent non-cancerous tissue samples were identified to carry out the analysis.

Validation of NOP58 Overexpression in HCC Tissues
To verify the overexpression of NOP58 in HCC tissues, we examined NOP58 expression in 371 HCC tissues and 50 normal, non-cancerous tissues. A significant overexpression of NOP58 in HCC was observed and compared with normal tissues (P < 0.001; Fig. 2a). Also, in normal tissues of GTEx combined with TCGA and TCGA corresponding tumor samples, NOP58 was significantly overexpressed (Fig. 2b). Further analysis of the expression of NOP58 in 50 pairs of HCC tissues and adjacent non-cancerous tissues showed that NOP58 was prominently overexpressed in HCC tissues (P < 0.001; Fig. 2c). The ROC curve analysis revealed an area under the curve of 0.904, which suggested that NOP58 might serve an important role in the development of HCC (Fig. 2d).

Validation of TCGA Database with External Database
The Oncomine database afforded one similarly published study [26]. We collected the expression values of HCC and normal group, analyzed by t test. Statistical analysis revealed that NOP58
expression was significantly elevated in HCC compared with the control group (Fig. 3).

Correlations Between NOP58 Expression and Clinical Characteristics in Patients with HCC

The results show that overexpression of NOP58 was correlated significantly with T stage ($P = 0.018$), pathologic stage ($P = 0.012$), tumor status ($P < 0.001$), histologic grade ($P < 0.001$), adjacent hepatic tissue inflammation ($P = 0.017$), AFP (ng/mL; $P < 0.001$), vascular invasion ($P = 0.002$), TP53 status ($P < 0.001$), race ($P = 0.019$), age ($P = 0.016$), weight ($P < 0.015$), and body mass index (BMI; $P = 0.030$), as shown in Fig. 4.

Chi-square test or Fisher’s exact test revealed that NOP58 was significantly correlated with T stage ($P = 0.003$), pathologic stage ($P = 0.003$), histologic grade ($P < 0.001$), adjacent hepatic tissue inflammation ($P = 0.011$), vascular invasion ($P = 0.024$), tumor status ($P = 0.031$), and TP53 status ($P < 0.001$). T test or Wilcoxon rank-sum test showed that NOP58 was significantly correlated with age ($P = 0.041$) and AFP level (ng/mL; $P < 0.001$; Table 1).

A univariate logistic regression of NOP58 TPM and the clinicopathological characteristics of HCC expression indicated that NOP58 significantly correlated with T stage ($P < 0.001$), pathologic stage ($P < 0.001$), histologic grade ($P < 0.001$), vascular invasion ($P = 0.003$), tumor status ($P = 0.003$), and TP53 status ($P < 0.001$; Table 2).
Identification of Prognostic Factors for HCC Prognosis

Cox regression model for univariate analysis of prognostic factors for OS revealed that high NOP58 expression levels were associated with worse OS (HR 1.950, 95% CI 1.362–2.790, \(P < 0.001\)), higher TM stage (T: HR 2.109, 95% CI 1.469–3.028; \(P < 0.001\)), M: HR 40.032, 95% CI 1.267–12.831, \(P = 0.018\), higher disease stage (HR 20.074, 95% CI 1.418–30.032, \(P < 0.001\)), and poor tumor status (HR 2.361, 95% CI 1.620–3.441, \(P < 0.001\); Table 3).

**Fig. 2** Correlation between NOP58 gene expression and tumor cells. **a, b** Differential expression analysis of patients with HCC (TCGA) and healthy tissue samples (TCGA or TCGA + GTEx) was performed by Wilcoxon rank sum test; **c** NOP58 gene expression in HCC tissue samples and paired paracancerous samples were analyzed by Wilcoxon signed rank sum tests; **d** ROC curves demonstrate the effectiveness of NOP58 in differentiating tumor from non-tumor cell: abscissa shows false positive rate and ordinate shows true positive rate.

Abbreviations: AUC, Area under curve; CI, Confidence interval; GTEx, Genotype-Tissue Expression; NOP58; Nucleolar protein 58; TCGA, The Cancer Genome Atlas database; TPM, Transcripts per million reads.
The prognostic factors included for multivariate Cox regression analysis were T stage ($P < 0.001$), M stage ($P = 0.018$), pathologic stage ($P < 0.001$), tumor status ($P < 0.001$), and NOP58 expression ($P < 0.001$). Furthermore, multivariate Cox regression analysis showed that tumor status ($P = 0.002$) and NOP58 gene expression ($P = 0.036$) were independent prognostic factors ($P < 0.05$) for OS (Table 3). These data indicate that NOP58 may be a prognostic factor, and increased NOP58 level is associated with poor OS.

Fig. 3 Validation of TCGA database with external database study

Fig. 4 Correlation of NOP58 gene expression with clinicopathological characteristics. a T stage; b pathologic stage; c histologic grade; d TP53 status; e AFP levels; f adjacent hepatic tissue inflammation; g vascular invasion; h tumor status; i BMI; j weight; k age; l race
Table 1  Correlation between *NOP58* expression and clinicopathologic features in the validation cohort

| Variable          | No. | *NOP58* expression |   |   |
|-------------------|-----|--------------------|---|---|
|                   |     | Low               | High |   |
| **T stage (%)**   |     |                   |     |   |
| T1                | 181 | 107 (58.5%)       | 74 (400.0%) | 0.003a |
| T2                | 94  | 36 (19.7%)        | 58 (31.4%)  |   |
| T3                | 80  | 33 (180.0%)       | 47 (25.4%)  |   |
| T4                | 13  | 7 (3.8%)          | 6 (3.2%)    |   |
| **N stage (%)**   |     |                   |     |   |
| N0                | 252 | 117 (99.2%)       | 135 (97.8%) | 0.627b |
| N1                | 4   | 1 (0.8%)          | 3 (2.2%)    |   |
| **M stage (%)**   |     |                   |     |   |
| M0                | 266 | 124 (98.4%)       | 142 (98.6%) | 1b |
| M1                | 4   | 2 (1.6%)          | 2 (1.4%)    |   |
| **Pathologic stage (%)** |     |                   |     |   |
| Stage I           | 171 | 99 (58.9%)        | 72 (40.2%)  | 0.003ab |
| Stage II          | 86  | 32 (190.0%)       | 54 (30.2%)  |   |
| Stage III         | 85  | 34 (20.2%)        | 51 (28.5%)  |   |
| Stage IV          | 5   | 3 (1.8%)          | 2 (1.1%)    |   |
| **Residual tumor (%)** |     |                   |     |   |
| R0                | 324 | 165 (94.8%)       | 159 (94.6%) | 0.902b |
| R1                | 17  | 8 (4.6%)          | 9 (5.4%)    |   |
| R2                | 1   | 1 (0.6%)          | 0 (0.0%)    |   |
| **Histologic grade (%)** |     |                   |     | < 0.001a |
| G1                | 55  | 40 (21.9%)        | 15 (8.2%)   |   |
| G2                | 177 | 98 (53.6%)        | 79 (43.2%)  |   |
| G3                | 122 | 40 (21.9%)        | 82 (44.8%)  |   |
| G4                | 12  | 5 (2.7%)          | 7 (3.8%)    |   |
| **Gender (%)**    |     |                   |     |   |
| Female            | 121 | 58 (31.2%)        | 63 (34.1%)  | 0.632 |
| Male              | 250 | 128 (68.8%)       | 122 (65.9%) |   |
| **Race (%)**      |     |                   |     |   |
| Asian             | 158 | 73 (410.0%)       | 85 (470.0%) | 0.333 |
| Black, African American | 17 | 7 (3.9%)          | 10 (5.5%)   |   |
| White             | 184 | 98 (55.1%)        | 86 (47.5%)  |   |
| Variable                                      | No. | NOP58 expression |     |     |
|-----------------------------------------------|-----|-----------------|-----|-----|
|                                               |     | Low             | High|     |
| Adjacent hepatic tissue inflammation (%)      |     |                 |     |     |
| Mild                                          | 99  | 42 (33.3%)      | 57  | 52.8%|
| None                                          | 117 | 73 (57.9%)      | 44  | 40.7%|
| Severe                                        | 18  | 11 (8.7%)       | 7   | 6.5% |
| Child–Pugh grade (%)                          |     |                 |     |     |
| A                                             | 217 | 120 (90.2%)     | 97  | 91.5%|
| B                                             | 21  | 12 (90.0%)      | 9   | 8.5% |
| C                                             | 1   | 1 (0.8%)        | 0   | 0.0% |
| Fibrosis Ishak score (%)                      |     |                 |     |     |
| 0                                             | 74  | 44 (38.3%)      | 30  | 30.9%|
| 1/2                                           | 31  | 14 (12.2%)      | 17  | 17.5%|
| 3/4                                           | 28  | 14 (12.2%)      | 14  | 14.4%|
| 5/6                                           | 79  | 43 (37.4%)      | 36  | 37.1%|
| Vascular invasion (%)                         |     |                 |     |     |
| No                                            | 206 | 116 (71.6%)     | 90  | 58.8%|
| Yes                                           | 109 | 46 (28.4%)      | 63  | 41.2%|
| Tumor status (%)                              |     |                 |     |     |
| Tumor free                                    | 201 | 111 (63.1%)     | 90  | 51.1%|
| With tumor                                    | 151 | 65 (36.9%)      | 86  | 48.9%|
| TP53 status (%)                               |     |                 |     |     |
| Mut                                           | 102 | 30 (16.5%)      | 72  | 40.9%|
| WT                                            | 256 | 152 (83.5%)     | 104 | 59.1%|
| Age (median [IQR])                            |     | 630.00 [540.00, 690.00] | 590.00 [510.00, 680.00] | 0.041<sup>a,c</sup> |
| Height (median [IQR])                         |     | 1680.00 [1610.00, 1750.00] | 1670.00 [1610.00, 1730.00] | 0.467<sup>c</sup> |
| Weight (median [IQR])                         |     | 71.50 [610.00, 85.75] | 690.00 [58.25, 78.75] | 0.071<sup>c</sup> |
| BMI (median [IQR])                            |     | 25.18 [22.12, 290.03] | 23.78 [21.28, 27.99] | 0.117<sup>c</sup> |
| AFP (ng/mL; median [IQR])                     |     | 70.00 [30.00, 41.50] | 400.00 [70.00, 1658.50] | <0.001<sup>a,c</sup> |
| Albumin (g/dL; median [IQR])                  |     | 40.00 [3.50, 4.30] | 40.00 [3.50, 4.30] | 0.6<sup>c</sup> |
The 10-year overall survival rates were significantly poor among patients with high NOP58 expression than those with low NOP58 expression (HR 1.95, 95% CI 1.36–2.79; \( P < 0.001 \) (Fig. 5a). The 10-year DSS rates in the high expression group were also significantly poorer than those in the low expression group (HR

### Table 1 continued

| Variable                               | No. | NOP58 expression | \( P \)  |
|----------------------------------------|-----|-----------------|--------|
|                                           | Low | High            |        |
| Prothrombin time (median [IQR])        | 1.10 [10.00, 9.30] | 1.10 [10.00, 8.80] | 0.816\(^{c}\) |

Categorical variables were analyzed by chi-square test; numeric variables were analyzed by \( t \) test. 

\( \text{AFP} \) alpha-fetoprotein, \( \text{IQR} \) interquartile range, \( \text{BMI} \) body mass index, \( \text{NOP58} \) nucleolar protein 58, \( \text{TP53} \) tumor protein 53, \( \text{WT} \) wild type. 

\(^{a}\) Statistically significant  
\(^{b}\) Fisher’s exact test  
\(^{c}\) Non-normal distribution; statistical analysis was performed using Wilcoxon rank sum test.

### Table 2 Univariate logistic regression of NOP58 TPM and clinicopathological characteristics

| Characteristics                                      | Odds ratio in NOP58 expression | Odds ratio | \( P \) value |
|------------------------------------------------------|--------------------------------|------------|---------------|
| T stage (T2 + T3 + T4 vs T1)                         | 368                            | 10.02 (10.01–10.03) | < 0.001* |
| N stage (N1 vs N0)                                   | 256                            | 10.01 (0.98–10.04) | 0.346  |
| M stage (M1 vs M0)                                   | 270                            | 10.02 (0.98–10.05) | 0.237  |
| Pathologic stage (stage II + stage III + stage IV vs stage I) | 347                            | 10.02 (10.01–10.03) | < 0.001* |
| Histologic grade (G3 + G4 vs G1 + G2)               | 366                            | 10.03 (10.02–10.04) | < 0.001* |
| Residual tumor (R1 + R2 vs R0)                      | 342                            | 10.01 (0.99–10.03) | 0.317  |
| Child–Pugh grade (B + C vs A)                        | 239                            | 10.00 (0.97–10.02) | 0.902  |
| Fibrosis Ishak score (1/2 + 3/4 + 5/6 vs 0)          | 212                            | 10.00 (0.99–10.02) | 0.483  |
| Adjacent hepatic tissue inflammation (mild + severe vs none) | 234                            | 10.01 (10.00–10.02) | 0.195  |
| Vascular invasion (yes vs no)                        | 315                            | 10.02 (10.01–10.03) | 0.003* |
| Tumor status (with tumor vs tumor free)              | 352                            | 10.01 (10.01–10.03) | 0.003* |
| TP53 status (Mut vs WT)                              | 358                            | 10.02 (10.01–10.03) | < 0.001* |

\( \text{AFP} \) alpha-fetoprotein, \( \text{BMI} \) body mass index, \( \text{IQR} \) interquartile range, \( \text{NOP58} \) nucleolar protein 58, \( \text{TP53} \) tumor protein 53, \( \text{WT} \) wild type.  

*Statistically significant.

### Survival Analysis

The 10-year overall survival rates were significantly poor among patients with high NOP58 expression than those with low NOP58 expression (HR 1.95, 95% CI 1.36–2.79; \( P < 0.001 \) (Fig. 5a). The 10-year DSS rates in the high expression group were also significantly poorer than those in the low expression group (HR
Table 3  Associations with overall survival and clinicopathologic characteristics in tcga patients using Cox regression analysis

| Clinicopathologic variable | Total (N) | HR (95% CI)     | P value |
|----------------------------|-----------|-----------------|---------|
| **Univariate regression analysis** |           |                 |         |
| T stage (T2 + T3 + T4 vs T1) | 367       | 2.109 (1.469–3.028) | < 0.001 |
| N stage (N1 vs N0)           | 256       | 20.004 (0.491–8.181) | 0.333   |
| M stage (M1 vs M0)           | 270       | 40.032 (1.267–12.831) | 0.018   |
| Pathologic stage (stage II + stage III + stage IV vs stage I) | 346 | 20.074 (1.418–30.032) | < 0.001 |
| Histologic grade (G3 + G4 vs G1 + G2) | 365 | 1.120 (0.781–1.606) | 0.539   |
| Residual tumor (R1 + R2 vs R0) | 341       | 1.571 (0.795–3.104) | 0.194   |
| Age (years; > 60 vs ≤ 60)    | 370       | 1.248 (0.880–1.768) | 0.214   |
| Gender (male vs female)      | 370       | 0.816 (0.573–1.163) | 0.26    |
| Weight (kg; > 70 vs ≤ 70)    | 343       | 0.916 (0.640–1.312) | 0.634   |
| Height (m; ≥ 170 vs < 170)   | 338       | 1.208 (0.833–1.753) | 0.319   |
| BMI (kg/m²; > 25 vs ≤ 25)    | 334       | 0.818 (0.563–1.186) | 0.289   |
| Race (white vs Asian + black/African American) | 358 | 1.245 (0.867–1.789) | 0.235   |
| Child–Pugh grade (B + C vs A) | 238       | 1.616 (0.797–3.275) | 0.183   |
| AFP (ng/mL; > 400 vs ≤ 400)  | 277       | 10.056 (0.646–1.727) | 0.827   |
| Albumin (g/dL; ≥ 3.5 vs < 3.5) | 296 | 0.921 (0.565–1.503) | 0.743   |
| Prothrombin time (> 4 vs ≤ 4) | 293       | 1.330 (0.877–20.015) | 0.179   |
| Fibrosis Ishak score (1/2 + 3/4 + 5/6 vs 0) | 211 | 0.779 (0.470–1.293) | 0.334   |
| Adjacent hepatic tissue inflammation (mild + severe vs none) | 233 | 1.228 (0.755–1.997) | 0.409   |
| Vascular invasion (yes vs no) | 314       | 1.348 (0.890–20.042) | 0.159   |
| Tumor status (with tumor vs tumor free) | 351 | 2.361 (1.620–3.441) | < 0.001 |
| TP53 status (Mut vs WT)       | 357       | 1.434 (0.972–2.115) | 0.069   |
| NOP58 (high vs low)           | 370       | 1.950 (1.362–2.790) | < 0.001 |
| **Multivariate regression analysis** |           |                 |         |
| T stage (T2 + T3 + T4 vs T1) | 367       | 0.834 (0.113–6.172) | 0.859   |
| M stage (M1 vs M0)           | 270       | 1.790 (0.427–7.506) | 0.426   |
| Pathologic stage (stage II + stage III + stage IV vs stage I) | 346 | 2.582 (0.336–19.840) | 0.362   |
| Tumor status (with tumor vs tumor free) | 351 | 2.236 (1.353–3.694) | 0.002   |
| TP53 status (Mut vs WT)       | 357       | 1.466 (0.870–2.468) | 0.15    |
| NOP58 (high vs low)           | 370       | 1.731 (10.037–2.890) | 0.036   |

*AFP* alpha-fetoprotein, *BMI* body mass index, *HR* hazard ratio, *NOP58* nucleolar protein 58, *TP53* tumor protein 53, *WT* wild type
2.17, 95% CI 1.37–3.44; \( P < 0.001 \) (Fig. 5b); similar results were observed in PFI analysis (HR 1.52, 95% CI 1.13–2.04; \( P = 0.006 \) (Fig. 5c).

Further we performed a subgroup analysis of the NOP58 expression groups stratified by inflammation, Ishak fibrosis score, vascular invasion, tumor status, vascular invasion, prothrombin and albumin. Patients with adjacent hepatic tissue inflammation had significantly better DSS (HR 3.41, 95% CI 1.26–9.25; \( P = 0.016 \) vs HR 1.57, 95% CI 0.65–3.81; \( P = 0.319 \)) and OS (HR 2.11, 95% CI 1.00–4.47; \( P = 0.05 \) vs HR 1.71, 95% CI 0.85–3.42; \( P = 0.13 \)) than those without inflammation. Similarly, the group with Ishak fibrosis score 1/2 and 3/4 and 5/6 had significant higher DSS (HR 2.59, 95% CI 1.06–6.36; \( P = 0.038 \) vs HR 2.40, 95% CI 0.87–6.59; \( P = 0.091 \)) and OS (HR 2.78, 95% CI 1.33–5.81; \( P = 0.007 \) vs HR 1.91, 95% CI 0.91–4.01; \( P = 0.087 \)) compared with Ishak fibrosis score 0. The vascular invasion group correlated significantly with better OS (HR 2.24, 95% CI 1.12–4.46; \( P = 0.022 \) vs HR 1.49, 95% CI 0.88–2.50; \( P = 0.138 \)) and PFI (HR 2.18, 95% CI 1.29–3.68; \( P = 0.004 \) vs HR 1.15, 95% CI 0.74–1.78; \( P = 0.531 \)) compared with the group without vascular invasion. Similarly, better DSS, OS, and PFI results were obtained in the group having tumor, albumin (g/dl) \( \geq 3.5 \), and prothrombin time > 4. These findings indicate that NOP58 plays a role in survival of patients with HCC by interacting with factors such as adjacent hepatic tissue inflammation, high Ishak fibrosis risk score, vascular invasion, with tumor state, albumin (g/dl) \( \geq 3.5 \), and prothrombin time > 4. However, as a result of the limitation in sample size during stratification, we could not analyze factors such as histologic grade, M stage, N stage, and pathologic stage (Fig. 6).

**NOP58-Related Signaling Pathways Based on GSEA**

On the basis of the analysis results of NOP58 co-expression genes, GSEA [27] was performed on low and high expression groups of NOP58 underlying the LIHC expression matrix derived from TCGA by the clusterProfiler package. Significant differences (FDR < 0.25, adjusted \( P \) value < 0.05) were demonstrated by GSEA in enrichment of MSigDB Collections (c2.all.v6.2.symbols.gmt). The results showed that the most significantly enriched signaling pathways include the cell cycle, mitotic G1/S phase, mitotic G2/M phase, Rb-1 pathway, M phase, and interleukin-10 (IL-10) signaling pathway, which indicates the potential role of NOP58 in the development of liver cancer. The details are shown in Fig. 7 and Table 4.
Fig. 6 Correlation between *NOP58* gene expression in high and low groups with (1) disease-specific survival, (2) overall survival, and (3) progression-free interval in patients with HCC from TCGA-LIHC database. **1A** Adjacent tissue inflammation (mild & severe vs none); **1B** albumin (g/dl) (≥ 3.5 vs < 3.5); **1C** fibrosis ishak score (1/2 & 3/4 & 5/6 vs 0); **1D** prothrombin time (≤ 4 vs > 4); **1E** with tumor vs tumor free. **2A** Adjacent tissue inflammation (mild and severe vs none); **2B** albumin (g/dl) (≥ 3.5 vs < 3.5); **2C** fibrosis ishak score (1/2 & 3/4 & 5/6 vs 0); **2D** prothrombin time (≤ 4 vs > 4); **2E** with tumor vs tumor free; **2F** vascular invasion (yes vs no). **3A** albumin (g/dl) (≥ 3.5 vs < 3.5); **3B** prothrombin time (≤ 4 vs > 4); **3C** with tumor vs tumor free; **3D** vascular invasion (yes vs no).
Correlation Between NOP58 Expression and Immune Infiltration

The marker genes of 24 kinds of immune cells were extracted as per a published method [24]. Spearman correlation was conducted to determine the association between the expression level (TPM) of NOP58 and immune cell infiltration level quantified by the ssGSEA method in the LIHC tumor microenvironment. The results showed that the NOP58 expression was positively correlated with Th2 cells, T helper cells, Tfh, and CD56bright NK cells, and negatively correlated with dendritic cells (DCs), Th17 cells, cytotoxic cells, neutrophils, and pDCs. Th2 cells were significantly positively correlated with NOP58 expression with Spearman R up to 0.428 with a P value < 0.001. Compared with the low expression cells, the enrichment score of Th2 cells was significantly higher in samples with NOP58 high expression (Fig. 8).

Fig. 7 Enrichment plots from the gene set enrichment analysis
Immunohistochemical Analysis

A comparison of normal and adjacent non-cancerous samples of HCC revealed that NOP58 was highly expressed in the cytoplasm and nucleus of HCC tissues compared with adjacent tissues, with statistically significant difference ($P < 0.05$) (Fig. 9).

DISCUSSION

Being one of the most prevalent malignant tumor in humans, the prognosis evaluation of HCC often poses great challenges [28]. It therefore becomes critical to investigate the underlying mechanisms for recurrence and metastasis of HCC [5, 29]. Early studies assumed that snoRNPs have a role only in the cellular housekeeping functions; however, recent evidence elucidated that the protein components of snoRNPs are involved in oncogenesis of various types of cancers [30–33]. Recently, Xu et al. showed that SNORD113-1 gene functions as a tumor suppressor in HCC. SNORD113-1 expression was significantly downregulated in HCC tumor cells compared to adjacent non-cancerous cells. Moreover, the results from a xenograft animal model provided direct evidence that SNORD113-1 can regulate HCC tumor cell growth and that the loss of SNORD113-1 gene function might be directly associated with tumorigenesis [34]. Another study outlined that overexpression of dyskerin gene, a member of the snoRNP family, might be an unfavorable prognostic factor in patients with HCC [35]. However, the role of the majority of snoRNPs in cancer remains unclear. Research on B16 mouse melanoma cells discovered that hNOP58 gene expression could be a potential marker for metastatic melanoma [22]. On the basis of this hypothesis, we carried out an investigation to understand the possible role of NOP58 gene expression in patients with HCC and its possible role as a prognostic biomarker for HCC.

In this study, we demonstrated that NOP58 was highly expressed in human HCC tissues, relative to adjacent non-cancerous tissues. In particular, according to the HCC data based on high-throughput RNA sequencing collected from TCGA database, NOP58 was significantly upregulated in HCC tissues compared to normal or adjacent normal tissues. Furthermore, NOP58

### Table 4 Gene sets enriched in phenotype high

| MSigDB collections | Gene sets name                                      | NES  | Adjusted $P$ value | FDR  |
|--------------------|----------------------------------------------------|------|--------------------|------|
| c2.cp.v70.0.symbols.gmt [Curated] | REACTOME_CELL_CYCLE_MITOTIC                         | 2.379 | 0.02              | 0.014|
|                     | REACTOME_MITOTIC_G1_G1_S_PHASES                    | 2.124 | 0.02              | 0.014|
|                     | REACTOME_MITOTIC_G2_G2_M_PHASES                    | 2.134 | 0.02              | 0.014|
|                     | PID_RB_IPATHWAY                                    | 1.9   | 0.022             | 0.015|
|                     | REACTOME_M_PHASE                                    | 2.175 | 0.02              | 0.014|
|                     | REACTOME_INTERLEUKIN_10_SIGNALING                   | 1.762 | 0.031             | 0.021|
|                     | KEGG_PATHWAYS_IN_CANCER                            | 1.651 | 0.02              | 0.014|
|                     | REACTOME_REGULATION_OF_TP53_ACTIVITY_THROUGH_PHOSPHORYLATION | 1.832 | 0.022             | 0.015|
|                     | PID_P53_DOWNSTREAM_PATHWAY                          | 1.646 | 0.024             | 0.016|

Gene sets with adjusted $P$ value of $< 0.05$ and FDR of $< 0.25$ are considered as significant

$NES$ normalized enrichment score, $FDR$ false discovery rate
overexpression in LIHC was significantly correlated with high clinical stage, advanced TM stage, advanced disease stage and tumor status, reduced survival time, and poor prognosis. The enrichment analysis to assess the function of NOP58 in LIHC also revealed that the cell cycle mitosis, mitotic G1/S phase, mitotic G2/M phase, Rb-1 pathway, M phase, IL-10 signaling pathways in cancer, regulation of TP53 activity through phosphorylation, and P53 downstream pathway were the most significant signaling pathways for HCC development. These pathways were reported to be significant contributors for cancer cell proliferation, invasion, and metastasis [36–38]. This data strongly indicates that NOP58 may not only be a potential prognostic biomarker but also a potential therapeutic target which affects the regulation of IL-10 signaling.

In addition, we used ssGSEA and Spearman correlation analysis to explore the association between NOP58 expression and immune infiltration levels in HCC. We found a strong correlation between NOP58 and dendritic cells, Th17 cells, and Th2 cells. Furthermore, the expression level of NOP58 was positively correlated with the infiltration level of Th2 cells. Elevation of NOP58 expression markedly

\[ \text{Correlation} \]

Abbreviations: CD8 T cells, cluster of differentiation 8 T-cells, DCs, Dendritic cells; aDCs, Activated DCs; iDCs, Immature DCs; NK cells, Natural killer cells; pDCs, Plasmacytoid dendritic cells; Th, Helper T cells; Treg, Regulatory T cells; Tgd, T gamma delta; Tcm, T central memory; Tem, T effector memory; Tfh, T follicular helper.

**Fig. 8** Correlation of NOP58 gene expression with the immune infiltration in the tumor microenvironment.  

- **a** Spearman correlation method was used to analyze the correlation between NOP58 and 24 immune cells;
- **b** Spearman correlation method was used to analyze the correlation between NOP58 expression and Th2 cells;
- **c** Th2 cells were at increased levels in the high NOP58 expression group analyzed by Wilcoxon rank sum test.
increased the Th2 cells, whereas levels of dendritic cells and Th17 cells had decreased. Thus, our findings indicate that NOP58 regulates the functions of Th2 cells in HCC via an unknown mechanism, which needs to be further explored.

It is well known that IL-10 is a pleiotropic cytokine predominantly produced by Th2 cells, macrophages, and B lymphocytes and can both stimulate and suppress the immune response [39]. Hsia et al. reported that IL-10 levels were frequently elevated in patients with HCC, thus playing a key role in the oncogenetic and metastatic ability of neoplasms, and might be related to disease prognosis in HCC [40]. Similar results were observed in this study, where we found that Th2 cells were significantly elevated in the NOP58 high expression group, and NOP58 may exhibit crosstalk with the IL-10 pathway. However, the specific role of NOP58 in

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Fig. 9 Immunohistochemistry of HCC tissues and normal non-cancerous cells for NOP58 expression

Abbreviations: IHC, Immunohistochemistry
the IL-10 signaling pathway is not yet clear with no detailed regulation mechanism between the two is reported.

Furthermore, we believe that NOP58 may be involved in the regulation of the cell cycle. Our findings revealed that the upregulation of NOP58 expression significantly correlated to the TP53 mutant group compared to that of the wild group. Previous studies suggested that wild TP53 can transcribe cyclin inhibitor p21\(^{\text{WAF1}}\), whereas the abnormal cell cycle caused by TP53 mutation would lead to uncontrolled growth of HCC [41, 42]. In the present study, GSEA revealed that the overexpression of NOP58 was primarily involved in the M phase, G1/S and G2/M phase of the cell cycle, Rb-1 pathway, regulation of TP53 activity through phosphorylation, and IL-10 pathway in LIHC. As previously described, cell cycle plays an important role in the development of HCC [43]. These findings suggest that cell cycle may have an important impact on the progression of HCC. However, the underlying regulatory mechanism by which NOP58 regulates cell cycle remains unknown. We speculate that NOP58 may affect cell cycle via IL-10 and Rb-1 signaling pathways. IL-10 can induce cell cycle activation by transitioning from G1 phase to G2/M phase, whereas the tumor suppressor gene Rb-1 encodes negative regulatory factors involved in cell cycle regulation.

In our study, for the first time we analyzed the expression level of NOP58 in both HCC cell lines and tissues, and elucidated that NOP58 was universally upregulated in HCC. However, there are a few limitations that warrant mention. Further immunohistochemistry along with animal study in larger sample size is needed to explore the functional mechanism of NOP58 in various signaling pathways. In addition, incomplete clinical data in the public database pose certain limitations. A larger prospective study should be conducted in the future and extended to multifunction omics.

CONCLUSIONS

Our research demonstrated that NOP58 overexpression was associated with poor survival and may serve as a potential biomarker for prognostication and therapeutic monitoring of patients with HCC. Moreover, there is a moderate to strong positive relationship between NOP58 expression level and infiltration level of Th2 cells. Moreover, cell cycle mitosis, mitotic G1/S phase, mitotic G2/M phase, Rb-1 pathway, M phase, IL-10 signaling, pathways in cancer, regulation of TP53 activity through phosphorylation, and P53 downstream pathway may be the key pathway regulated by NOP58 in patients with HCC. Further studies need to explore the underlying mechanisms and verify our findings in the future.

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**Compliance with Ethics Guidelines.** All datasets were from databases of de-identified patient data and so ethics committee approval was not required.

**Data Availability.** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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