Novel biomarker NEDD1 and its analysis in hepatocellular carcinoma

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Abstract

Background: NEDD1 (NEDD1 Gamma-Tubulin Ring Complex Targeting Factor) plays a crucial impact in regulating cell cycle and the development of scirrhou gastric cancer. However, the role of NEDD1 hasn't been reported in hepatocellular carcinoma (HCC) so far. The aim of this research is to explore the role of NEDD1 on the development and prognosis of HCC.

Methods: All methods were performed in accordance with the relevant guidelines and regulations. HCC-related data were download from The Cancer Genome Atlas (TCGA) database. Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and gene set enrichment analysis (GSEA) were conducted by the LinkedOmics database.

Results: The expression of NEDD1 has significant difference between tumor and adjacent normal tissues in HCC ($P = 0.01$). We also found that NEDD1 was an independent risk factor in HCC patients (HR 1.643, 95%CI 1.125–2.398; $P = 0.01$). The study also demonstrated that NEDD1 expression was significantly relevant to the expression of several immune checkpoint genes, including CTLA-4, PD-L1 and PD-1. GSEA revealed that Cell cycle, MicroRNAs in cancer and Ribosome pathways were significantly enriched in NEDD1 overexpression phenotype. By integrating NEDD1 with other relevant factors, we constructed the prognostic nomogram to help the improvement of the prognosis for patients with HCC. The data from the International Cancer Genome Consortium (ICGC) database were used as an independent external validation of our prognostic model.

Conclusion: The expression level of NEDD1 was negatively correlated to the prognosis of HCC patients and it may be a promising therapeutic target of HCC, which probably be able to predict the efficacy of immunotherapy for HCC patients.

Introduction

HCC constitutes approximately 90% of primary liver cancer worldwide¹, which ranks fourth leading cause of cancer-related mortality and sixth among cancer diagnoses². Despite the development of clinical managements of HCC, the prognosis of patients with HCC is dismal. The overall survival (OS) of HCC patients varies around the world, but it is still rather low with the 5-year survival rate 14.1% in China at present³. With the exploration of the treatment of HCC, the research of multikinase inhibitors combined with immune checkpoint inhibitors for patients at advanced stages has gradually become a hot spot. Additionally, atezolizumab plus bevacizumab was admitted for the first-line treatment in unresectable HCC on June 1, 2020 by Food and Drug Administration (FDA) because the combination therapy can extraordinarily prolong progression free survival (PFS) than sorafenib. However, the PFS is 6.8 months (5.7-8.3) for atezolizumab plus bevacizumab in unresectable HCC, which is still far from satisfaction. Unfortunately, there are few molecular classifications predicting progression or recurrence of HCC⁴. Consequently, it is urgently demanded to investigate robust prognostic indicator and promising targets for therapy of HCC patients.

NEDD1, also known as GCP-WD, a crucial component in controlling gamma-tubulin levels at the mammalian centrosome, promotes initiation of mitosis in human beings⁵. Previous studies indicated that NEDD1 probably be directly associated with cell cycle regulation^{6,7}. Cell cycle has been reported to play a crucial impact in the development of HCC^{8,9}, NEDD1 could be a potential novel target to inhibit cell proliferation in HCC. Previous report has also demonstrated that small interfering RNA (siRNA) targeting NEDD1 is able to reduce cell numbers, including cervix carcinoma cells HeLa, prostate carcinoma cells DU145, colon carcinoma cells DLD-1, ovarian adenocarcinoma cells SKOV-3, breast carcinoma cells MDAMB-231, pancreas adenocarcinoma cells BxPc-3 and lung carcinoma cells A549 in vitro, which reveals that NEDD1 is a potential therapeutic target for lots of cancer types⁷. Besides, in the scirrhous gastric cancer model mice, intraperitoneal delivery of a siRNA against NEDD1 can extend its survival¹⁰. However, there has not been a report analyzing the clinical significance of NEDD1 in HCC so far. Our findings indicates that NEDD1 play an essential impact on the development of HCC.

Materials & Methods

Data source and processing

The dataset of LIHC patients were downloaded from the TCGA database (<https://cancergenome.nih.gov/>), at the level 3 HTSeq-FPKM format. 373 HCC cases and 50 patients with paired normal tissues specimens were enrolled for analysis. Since complete clinical information on the patient is required to build the nomogram, we followed up by screening the patients, excluding cases with missing or deficient data on age, gender and OS. Meanwhile, data was transformed to TPM (transcripts per million reads), and the amount of normal liver tissue in TCGA was not enough, so we used the UCSC XENA database (<https://xenabrowser.net/datapages/>) for differential expression analysis of NEDD1 in addition to TCGA data for pairwise difference analysis¹¹.

Assessment of immune cell infiltration

To analyze the association of NEDD1 with the infiltrating abundances of immune cells in HCC, the associated data about the immune infiltration levels were downloaded from tumour immune assessment resource (TIMER) (<https://cistrome.shinyapps.io/timer/>)¹². Likewise, a single-sample gene set enrichment analysis (ssGSEA) was conducted to quantify the abundances of 24 categories of immune infiltration cells in tumor specimens¹³. We calculated normalized enrichment score (NES) by ssGSEA, which was employed in the “GSVA” and “GSEABase” R package¹⁴ (version3.14.3, <https://www.bioconductor.org/packages/release/bioc/html/GSVA.html>). Spearman rank correlation method was conducted to investigate the relevance of NEDD1 expression with the infiltration levels. We also employed the GEPIA website (Gene Expression Profiling Interactive Analysis) to assess the correlation of NEDD1 with immune checkpoint genes¹⁵.

Enrichment Analysis.

Through the LinkedOmics database, we investigate the differentially expressed genes associated with NEDD1¹⁶. Pearson correlation coefficient was employed to test for the differences. Gene set enrichment analysis (GSEA) was conducted to assess KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment¹⁷ and GO (Gene Ontology) analysis, which is composed of cellular component (CC), biological process (BP) and molecular function (MF)¹⁸. GSEA was employed to explore whether a priori defined group of genes have statistically significant and concordant differences between two biological status¹⁹ (20). In this research, GSEA produced a list of all genes, which was generated based on their association with NEDD1 expression. The R package “clusterProfiler” (version 4.1.0) was employed to conduct GSEA in high- and low-NEDD1 expression groups²⁰. For each analysis, gene set permutation was performed 1000 times. Meanwhile, Gen sets with normalized enrichment score (NES) 1, P 0.05, and false discovery rate (FDR) q value 0.25 were judged as enriched significantly.

Construction and validation of prognostic nomogram in HCC patients

We constructed the nomogram, a quantifiable analysis tool which integrate the value of NEDD1 expression with other variables, to infer the mortality rate of individual HCC patients. Harrell's concordance index (C-index) was measured to assess the differentiation performance of the constructed nomogram. Meanwhile, we plotted the calibration curves to assess the accuracy of nomograms by comparing the predicted values of the nomogram with the observed actual survival rates. R package “survival” and “rms” were conducted to plot the nomogram and calibration curves.

Statistical Analysis

Statistical analyses were performed by R-4.1.0 (<http://www.r-project.org>).. Wilcoxon rank sum tests was employed to assess NEDD1 expression in tumor and normal groups. Meanwhile, Wilcoxon signed-rank tests was conducted to distinguish NEDD1 expression in paired specimens. We employed the Kaplan–Meier method to investigate the relevance of NEDD1 expression and OS in Pan-cancer analysis. Cox regression analysis was conducted to explore the role of NEDD1 and other associated varies on survival. We chose a univariate analysis p-value of less than 0.01 as the threshold for inclusion in the multivariate analysis. The analysis was done through R package “survival” and visualization was done through R package “Forestplot”. P 0.05 indicated statistically significant, if not specified. Time-dependent ROC curves was plotted by R package “survivalROC”²¹. R package “ggplot2” was employed to visualize the results.

Results

The differential expression of NEDD1 in HCC

We assessed the differential expression analysis of NEDD1 in HCC patients through the Wilcoxon rank-sum. The results indicated that NEDD1 expression was obviously higher in LIHC, LUSC, et al. than in normal tissue by comparing NEDD1 expression in normal samples of Genotype-Tissue Expression (GTEx) and TCGA, and tumor samples of TCGA (Figs. 1A,1C). We further demonstrated our findings in paired samples and found that NEDD1 expression also higher in CHOL, KICH, LIHC et al. (Fig. 1B). The results showed that NEDD1 was closely associated with cancer.

The relationship between NEDD1 expression and HCC prognosis

Kaplan-Meier survival curve was conducted to explore the relevance of NEDD1 expression and OS of patients in pan cancer, which indicated that NEDD1 was relevant to poor OS in HCC($P=0.002$), Kidney renal clear cell carcinoma($P=0.046$) and lung adenocarcinoma ($P=0.020$) (Fig. 2). Subsequently, forest plot regarding OS of the univariate and multivariate Cox regression analysis in HCC demonstrated that high NEDD1 expression was an independent risk indicator for HCC patients (HR 1.643, 95%CI 1.125–2.398; $P = 0.01$) (Fig. 3). The finding indicated that NEDD1 was significantly related to HCC prognosis and probably be a novel prognostic marker for HCC.

Immune infiltration analysis of NEDD1 in HCC patients

Using TIMER, we assessed the association of NEDD1 expression with the abundance of immune infiltration in HCC. The results revealed that tumor purity is not directly associated with the NEDD1 expression, but a positive relevance existed between NEDD1 expression and the abundance of infiltrating B cells($r = 0.36$, $P = 6.08e-12$), CD8+T cells($r = 0.334$, $P = 2.41e-10$), CD4+ T cells ($r = 0.471$, $P = 2.27e-20$), Macrophages ($r = 0.548$, $P = 3.90e-28$), Neutrophils ($r = 0.579$, $P = 2.62e-32$), and DCs ($r = 0.492$, $P = 3.93e-22$) in HCC(Fig. 4A). Meanwhile, the results revealed that the immune infiltrating levels were not directly relevant to OS of HCC patients (Fig. 4B). Subsequently, Spearman correlation was performed to explore the relevance of NEDD1 with the immune cells' infiltration levels quantified by ssGSEA method. Our results showed that there were positive association of NEDD1 expression with Th2 cells and Th cells, and the negative correlation of NEDD1 with cytotoxic cells, pDC and DC was found in Fig. 4C. As show in Fig. 4D and Fig. 4E, the level of NEDD1 expression were significantly positively related to Th cells ($r=0.440$, $P = 0.001$) and Th2 cells ($r=0.510$, $P = 0.001$).

Previous studies suggested that immune checkpoint inhibitors are a promising treatment modality for the effective treatment of cancer (23). We used GEPIA to explore the relevance of NEDD1 with the immune checkpoint genes, which indicated that NEDD1 was closely associated with immune checkpoint genes in pan cancer (Fig. 5A). Meanwhile, our findings revealed that NEDD1 expression was related to the

expression of CTLA4 ($r=0.260$, $P=0.001$), CD274(PD-L1) ($r=0.400$, $P=0.001$) and PTCD1(PD-1) ($r=0.270$, $P=0.001$). (Fig. 5B,5C)

GO and KEGG analyses of NEDD1

To analyze the biological function of NEDD1, we employed the LinkedOmics database to explore the co-expressed genes of NEDD1 in LIHC from TCGA, firstly (Fig. 6A). It indicated that the expression of PPP1R12A, GTE3, et al were positively associated with NEDD1 in Fig. 6B. On the contrary, ZNHIT1, DCI, et al were negatively associated with NEDD1 in Fig. 6C. Subsequently, based on GSEA in LinkedOmics, we conducted GO analysis. The results demonstrated that NEDD1 was significantly associated with the Cell cycle, Homologous recombination and MicroRNAs in cancer signaling pathways (Fig. 6D). Meanwhile, NEDD1 plays an important part in the process of histone binding, helicase activity and is located in chromosomal region and microtubule organizing center part (Figs. 6E,6F).

In addition, on the basis of GSEA, we performed KEGG analysis demonstrating that NEDD1 expression is positively associated with the following signaling pathways, including Cell cycle, MicroRNAs in cancer and Ribosome. It also showed that NEDD1 was negatively correlated with Ribosome pathway (Figs. 7A-7D). These results demonstrated that NEDD1 played a significant impact in HCC development.

Construction and validation of prognostic nomogram in HCC patients

Further analysis revealed the diagnostic value of NEDD1 in OS and disease-specific survival (DSS) of HCC. The results indicated that the AUC values of 1-year OS, 3-year OS and 5-year OS were 0.688, 0.629 and 0.628, respectively. It also suggested that the AUC values of 1-year DSS, 3-year DSS and 5-year DSS were 0.722, 0.658 and 0.634, respectively (Figs. 8C,8D). Thus, the nomogram was built to estimate the 1-, 3-, and 5-year survival probability by integrating NEDD1 expression with relevant variables, including age, gender, tumor status and other clinical factors (Fig. 8A). There are 373 original data, 208 cases with missing variable information, and the final number of enrolled samples is 165. The consistency test (Concordance, C-index) of the nomogram was 0.712 (0.663-0.761). As well, the calibration curve illustrated that there was excellent agreement between the predicted and actual survival in the prognostic nomogram, suggesting that it had good predictive value. (Fig. 8B).

Subsequently, primary liver cancer data from ICGC was employed as an external validation of the nomogram. As shown Figs.9A-9C, it demonstrated that in the ICGC database, this model has good predictive power and can clearly distinguish between low, medium and high risks HCC patients.

Discussion

Because of the absence of valid approaches to early diagnosis for HCC patients, almost 70% HCC patients are diagnosed at an unresectable state^{1, 22, 23}, leading to limited treatment options and a very poor prognosis. It is of tremendous significance to make an early diagnosis and effectively predict the prognosis for improving the overall survival in HCC. Consequently, it is urgently needed to investigate potential biomarkers for diagnosis and prognosis, and a novel therapeutic target of HCC patients.

NEDD1, the gamma-tubulin ring complex targeting factor, plays an essential impact in regulating cell cycle. Moreover, it has been proved that appropriate doses of siRNA against NEDD1 can affect numerous types of tumor cells growth in vitro and significantly prolong the overall survival of scirrhus gastric cancer model mice in in vivo^{7, 10}. Thus, NEDD1 probably be a promising target for HCC therapy. However, according to our knowledge, the potential impact of NEDD1 in HCC is still insufficient.

In this research, NEDD1 expression profiles and survival data were downloaded from TCGA to analyze the impact of NEDD1 in HCC. The results indicated that NEDD1 expression was higher in human HCC tissues than that in adjacent normal tissues. Meanwhile, overexpression of NEDD1 was considered to be an independent prognostic factor for poor OS by Kaplan-Meier survival curve and multivariable analysis in HCC patients, which indicated that NEDD1 could be used to predict the prognostic of HCC and an alternative therapy target for HCC.

Additionally, we found positive correlations between NEDD1 expression and the abundance of infiltrating B cells ($r = 0.36$, $P = 6.08e-12$), CD8+T cells ($r = 0.334$, $P = 2.41e-10$), CD4+T cells ($r = 0.471$, $P = 2.27e-20$), Macrophages ($r = 0.548$, $P = 3.90e-28$), Neutrophils ($r = 0.579$, $P = 2.62e-32$), and DCs ($r = 0.492$, $P = 3.93e-22$) in HCC. However, our study demonstrated that the relevance between immune cell infiltration and OS in HCC patients was not statistically significant, and that NEDD1 was negatively associated with OS in patients with HCC. This suggested that NEDD1 was not responsible for the poor prognosis of patients with HCC by affecting the abundances of infiltrating immune cells in the tumor. Consistent with us, it has been reported that HCC immune microenvironment has different impacts on the prognostic of HCC because of its heterogeneity²⁴. Meanwhile, Spearman correlation analysis and ssGSEA were conducted to detect the connection of NEDD1 expression with immune cells' infiltrating levels in HCC patients. As shown in Fig. 4D and 4E, Th2 cells and Th cells were positively related with NEDD1 expression. However, how NEDD1 regulates the abundance of immune cells in HCC is still unknown, which requires a further exploration. Our research also demonstrated that NEDD1 expression was significantly relevant to the expression of several immune-related genes, including CTLA-4, PD-L1 and PD-1. As immune checkpoint inhibitors are increasingly used in the management of unresectable HCC, there is an urgent need for molecules that predict their therapeutic efficacy²⁵. Our findings indicated that NEDD1 was most likely a predictor of the efficacy of immune checkpoint inhibitor therapy.

Consistent with previous studies^{5, 7}, GO and KEGG analyses indicated that NEDD1 was involved in cell cycle regulation and microtubule protein composition. Previous reports have suggested that the cell cycle plays a crucial impact in the development of HCC and we are able to ameliorate the prognostic of HCC patients by regulating hepatoma cell proliferation negatively via affecting the key biomarkers in the cell

cycle regulation, including PCK1, MCM6, PKM2 and others^{26–29}. Through the Linkedomics database we analyzed related genes co-expressed with NEDD1. The results revealed that NEDD1 was significantly relevant to PPP1R12A, GTE3, ZNHIT1, DCI, et al. PPP1R12A, also known as MYPT1, which is a member of the myosin family phosphatase-targeting protein (MYPT) family and regulates smooth muscle contraction^{30,31}. It has been reported that PPP1R12A is closely associated with processes, such as cell cycle³², development³³, migration and cell adhesion³⁴. A study by Xiao Zheng et al. also indicated that circPPP1R12A-encoded proteins can promote colon cancer development and metastasis³⁵. All of this indicated that PPP1R12A might play a crucial impact in the development of HCC. In turn, it has been demonstrated that NEDD1 played an important part in HCC development, but higher quality evidence was needed for validation.

Finally, we established a nomogram, for there wasn't a nomogram for HCC by integrating NEDD1 expression value with clinical characteristics for now. On the one hand, it helps to more precisely estimate the survival risk of individual patients; on the other hand, it helps clinicians guide patients' assessment and therapeutic decision-making³⁶. This predictive model is desired for providing help to promote the individualized therapy of HCC patients.

Conclusions

In conclusion, this study is the first to report the role of NEDD1 in HCC, suggesting that NEDD1 can be used to predict HCC prognostic and be a potential target therapeutic molecule. Besides, NEDD1 probably be able to predict the therapeutic efficacy of immune checkpoint inhibitors for hepatocellular carcinoma.

Abbreviations

NEDD1: NEDD1 Gamma-Tubulin Ring Complex Targeting Factor; TCGA: The Cancer Genome Atlas; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes. FDA: Food and Drug Administration; OS: overall survival. GTEx: The Genotype-Tissue Expression; ROC curve: Receiver Operating Characteristic curve; AUC: Areas under curve; ICGC: International Cancer Genome Consortium

Declarations

Ethics Approval and Consent to Participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

All data included in this study are available including The Cancer Genome Atlas (TCGA, <https://cancergenome.nih.gov/>), International Cancer Genome Consortium (ICGC, <https://dcc.icgc.org/>) and The Genotype-Tissue Expression (GTEx <https://gtexportal.org/home/>) portal.

Competing interests:

The authors declare that they have no competing interests.

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Authors' Contribution:

DXP, YJH, SX and LKW collected the information and drafted the initial manuscript. RPW, XGY and MSY participated in the discussion of the manuscript. DXP, YJH, MSY, HY and ZXM conceived and designed the study, performed data analysis, and wrote the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Figures

Figure 1

Analysis of differential expression of NEDD1 in pan-cancer A. Differential expression of NEDD1 in pan-cancer by using the data from TCGA and GTEx databases B. Differential expression of NEDD1 in pan-cancer by using the matched samples data from TCGA database C. Differential expression of NEDD1 in pan-cancer by using the unpaired samples data from TCGA database.

Figure 2

Kaplan-Meier Plot analysis of NEDD1 in cancers with differential expression

Figure 3

Forest plot of univariate and multivariate analyses of NEDD1 in HCC. A. Univariate analysis of NEDD1 in HCC B. Multivariate analysis of NEDD1 in HCC.

Figure 4

Immune infiltration of NEDD1 in HCC. A. Correlation of NEDD1 with 6 types of immune cells by using TiMER database B. Visualization of the survival differences by using Kaplan-Meier plots for immune infiltrates and NEDD1 C. Correlation of NEDD1 with 24 types of immune cells by using ssGSEA method D. Correlation of NEDD1 and Th2 cell expression in HCC E. D. Correlation of NEDD1 and T helper cell expression in HCC.

Figure 5

Correlation analysis of NEDD1 with immune checkpoint genes. A. Correlation analysis of NEDD1 with common immune checkpoint genes in pan-cancer B-D. Correlation analysis of NEDD1 with CTLA4, PD-L1, PD1 in HCC.

Figure 6

Co-expression analysis with NEDD1-related genes and GO analysis. A. Volcanic map of NEDD1-related genes B. Heatmap of genes positively associated with NEDD1 C. Heatmap of genes negatively associated with NEDD1 D. Biological process category of GO analysis E. Molecular function category of GO analysis F. Cellular component category of GO analysis.

Figure 7

KEGG pathway and GSEA. A. KEGG pathway analysis. B-D. GSEA of NEDD1 in Cell cycle, MicroRNAs in cancer, Ribosome pathways.

Figure 8

The prognostic analysis of NEDD1 and construction of prognostic model in HCC patients. A. Construction of nomogram by integrating common clinical indicators and the expression level of NEDD1 B. 1, 3, 5-years calibration curves C. Time-dependent ROC curve of NEDD1 in HCC patients with OS as the outcome endpoint D. Time-dependent ROC curve of NEDD1 in HCC patients with DSS as the outcome endpoint.

Figure 9

Validation of the prognostic model with the data from ICGC database. A. Risk score chart of the expression level of NEDD1 in HCC patients B. KM-plot analysis C. Time-dependent ROC curve analysis.