Am J Clin Exp Immunol 2024;13(1):26-34
www.ajcei.us /ISSN:2164-7712/AJCEI0153601

Original Article

Exploring the role of NAA40 in immune infiltrates and prognostic prediction in hepatocellular carcinoma

Tong Zhou[1,2], Jun Cao[1], Qingqin Tang[1], Jieyu Jin[1], Yuting Liang[1], Bin Feng[1]

[1]*Center for Clinical Laboratory, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China;*
[2]*Medical College of Soochow University, Suzhou, Jiangsu, China*

Received September 24, 2023; Accepted January 26, 2024; Epub February 25, 2024; Published February 28, 2024

Abstract: NAA40 belongs to the N-terminal acetyltransferase (NATs) family, responsible for protein N-terminal modification, and it exerts crucial roles across various cancers. However, its impact on patient prognosis and immune infiltration in hepatocellular carcinoma (HCC) remains elusive. To address this, our study delved into the comprehensive analysis of NAA40 in the context of cancer. Our pan-cancer analysis unveiled elevated NAA40 expression in multiple tumor types, including BLCA, BRCA, CHOL, COAD, ESCA, HNSC, LIHC, LUAD, LUSC, STAD, and THCA. Additionally, through a comprehensive examination across various cancer types within TCGA, we discovered that high NAA40 gene expression correlated with poor prognosis in HCC, pointing toward its role in promoting oncogenesis. Further investigation illuminated the association of increased NAA40 expression with T stage, pathologic stage, tumor status, and histologic grade. Interestingly, we noted a significant inverse correlation between NAA40 expression and the infiltration levels of immune cells, such as DC cells, neutrophils, NK cells, and T cells, in liver cancer. This observation underpins the hypothesis that NAA40 influences HCC development by modulating immune cell infiltration. Functional enrichment analysis provided valuable insights into the pathways influenced by NAA40. Enriched pathways encompassed oxidative phosphorylation, xenobiotic metabolism, bile acid metabolism, fatty acid metabolism, G2M checkpoint, and E2F targets. These findings collectively position NAA40 as a potential biomarker for prognostic prediction and monitoring the effects of immunotherapy in HCC.

Keywords: Hepatocellular carcinoma, NAA40, immune infiltrates, prognosis

Introduction

In the realm of global cancer statistics, Hepatocellular carcinoma (HCC) stands out as a prominent malignant tumor, exhibiting a distressing surge in mortality rates in recent decades, with a survival rate of merely 21% [1]. Despite advancements in the diagnosis and surveillance of HCC, driven by novel biomarkers and tailored screening recommendations based on the underlying causes of HCC2, the challenge persists [2]. Late-stage diagnosis and chemotherapy resistance render HCC vulnerable to recurrence and metastasis, resulting in unsatisfactory clinical outcomes [3]. Consequently, the imperative to identify novel molecular targets and enhance current treatment strategies is paramount.

NAA40, also known as NatD or Nat4, assumes a significant role within the family of N-terminal acetyltransferases (NATs). This enzyme catalyzes the addition of acetyl groups to the primary α-amino group of proteins' N-terminal residues [4]. The modification of protein N-terminal acetylation exerts broad influence across biological functions, encompassing protein-protein interactions, protein complex formation, apoptosis, rDNA transcriptional regulation, as well as protein subcellular localization and degradation [5]. Intriguingly, NatD's impact extends to histone H4 N-terminal acetylation at the slug promoter, eliciting epithelial-to-mesenchymal transition (EMT) in lung cancer cells [6]. This underscores NatD's role as a pivotal epigenetic regulator of cellular invasion. Elevated levels of NA440 mRNA are observed across various tumor types, notably more pronounced in HCC patients. This elevated expression correlates with patient survival and suggests a potential mechanism for promoting hepatocellular carcinoma progression through the inactivation of

the oncogene p53 [7-9]. Despite these insights into NA440's significance within tumors, the clinical implications of NA440 expression in pan-cancer settings, its relation to prognosis, its role in HCC, and the associated pathogenic mechanisms remain unclear.

This study embarks on a multifaceted investigation. It seeks to unravel the intricate relationship between NAA40 expression and the clinical prognosis of HCC. Moreover, it delves into the role of immune cell infiltration within the context of HCC through a bioinformatics lens. The overarching goal is to unearth the molecular mechanisms that underlie these phenomena. This endeavor, in turn, holds promise not only as a novel indicator but also as a potential therapeutic target.

Materials and methods

Data collection and processing

RNA-seq expression data and clinical records were downloaded from the Cancer Genome Atlas (TCGA) database and the Genotype Tissue Expression Project (GTEx). The utilization of these database was pivotal in examining the expression of NA440 in hepatocellular carcinoma (HCC) in this study.

Analysis of differences between high and low NA440 expression groups

HCC patients within the TCGA dataset were categorized into high and low NAA40 expression groups by the mean expression score. Differential expression analysis of genes (DEGs) between these groups was performed using the R package DESeq2. The criteria for defining DEGs included a significance threshold of adjusted *p*-value <0.05 and |log2-fold-change (FC)| >1.

Functional enrichment analysis

Enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), were conducted for the identified DEGs. Additionally, Gene set enrichment analysis (GSEA) was employed to assess statistically concordant differences between groups and explore the functions of NAA40 based on all genes. A significance threshold of adjusted P<0.05 was applied in these analyses.

Immune infiltration analysis

Utilizing ssGSEA facilitated by the R package-GSVA [1.46.0] [10], our study employed the immune cell markers from the Immunity article [11] to calculate the corresponding immune infiltration for 24 immune cell types. The differences in the level of immune infiltration were compared using the Wilcoxon rank-sum test.

Survival analysis

Survival outcomes were assessed using Kaplan-Meier curves along with the log-rank test. The impact of clinical variables on patient outcome were evaluated through univariate and multivariate Cox regression analyses, with a significance level set at P<0.05. To visually represent the results, a forest plot was generated using the R package ggplot2 in version 4.2.1.

Statistical methods

Data were analyzed using R (v.4.2.1). Differences between non-paired and paired tissues were compared using the Wilcoxon rank-sum test or paired sample t-test as appropriate. Both of tests are two-sided P<0.05 was the threshold of significance.

Results

Higher expression of NAA40 in HCC than in paracancerous tissues

The pan-cancer analysis results revealed elevated NAA40 expression in various tumor types, including BLCA, BRCA, CHOL, COAD, ESCA, HNSC, LIHC, LUAD, LUSC, STAD, and THCA (**Figure 1A**). This observation was substantiated by data from the TCGA and GTEx databases, indicating high expression across multiple tumor types, such as ACC, BLCA, CHOL, COAD, DLBC, ESCA, GBM, HNSC, LAML, LGG, OV, PAAD, PRAD, READ, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS (**Figure 1B**).

Moreover, in HCC, NAA40 demonstrated significantly higher expression levels compared to normal liver tissues (**Figure 1C**). This pattern remained consistent within paired HCC tissues, where NAA40 expression was elevated (**Figure 1D**). Furthermore, the diagnostic potential of NAA40 expression was evaluated through the

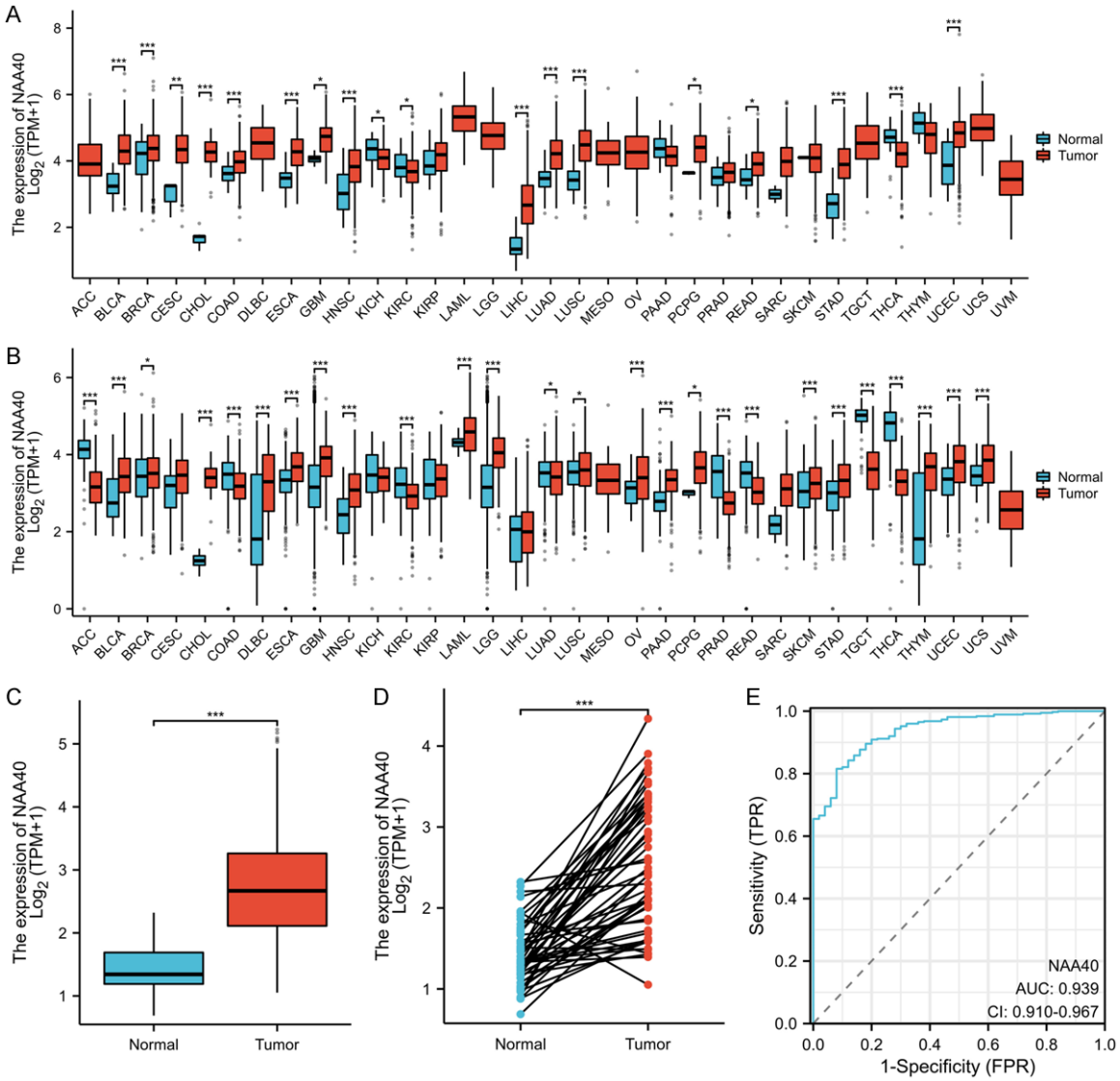


Figure 1. Higher expression of NAA40 in HCC than in noncancerous tissues. (A) NAA40 expression levels in 33 types of cancer tissues and normal tissues in TCGA databases. (B) NAA40 expression levels in 33 types of cancer tissues and normal tissues in TCGA and GTEx databases. Based on TCGA dataset. Expression of NAA40 (C) in HCC and non-matched normal tissues, (D) in HCC and matched normal tissues. (E) The ROC curve of diagnosis to distinguish tumor from normal tissue.

ROC curve, demonstrating its ability to accurately discriminate between tumor and normal tissue, as indicated by an area under the curve (AUC) of 0.939 (95% confidence interval [CI] =0.910-0.967) (**Figure 1E**).

NAA40 expression correlated with prognosis

The Cox analysis-based forest plot illustrating overall survival (OS) reveals a notable relationship between OS prognosis across various cancer types. Specifically, elevated gene expression is linked to a poorer prognosis in hepatocellular carcinoma (HCC) (P=0.0019, HR=1.736, 95% CI=1.22-2.46) (**Figure 2A**).

To assess the impact of NAA40 expression, patients were categorized into high and low expression groups based on the mean expression level. The correlation between NAA40 expression and HCC patient prognosis was evaluated through Kaplan-Meier survival curves. Notably, higher NAA40 expression in HCC cases exhibited a significant association with unfavorable outcomes. This was evident in poorer overall survival (hazard ratio [HR] =2.36,

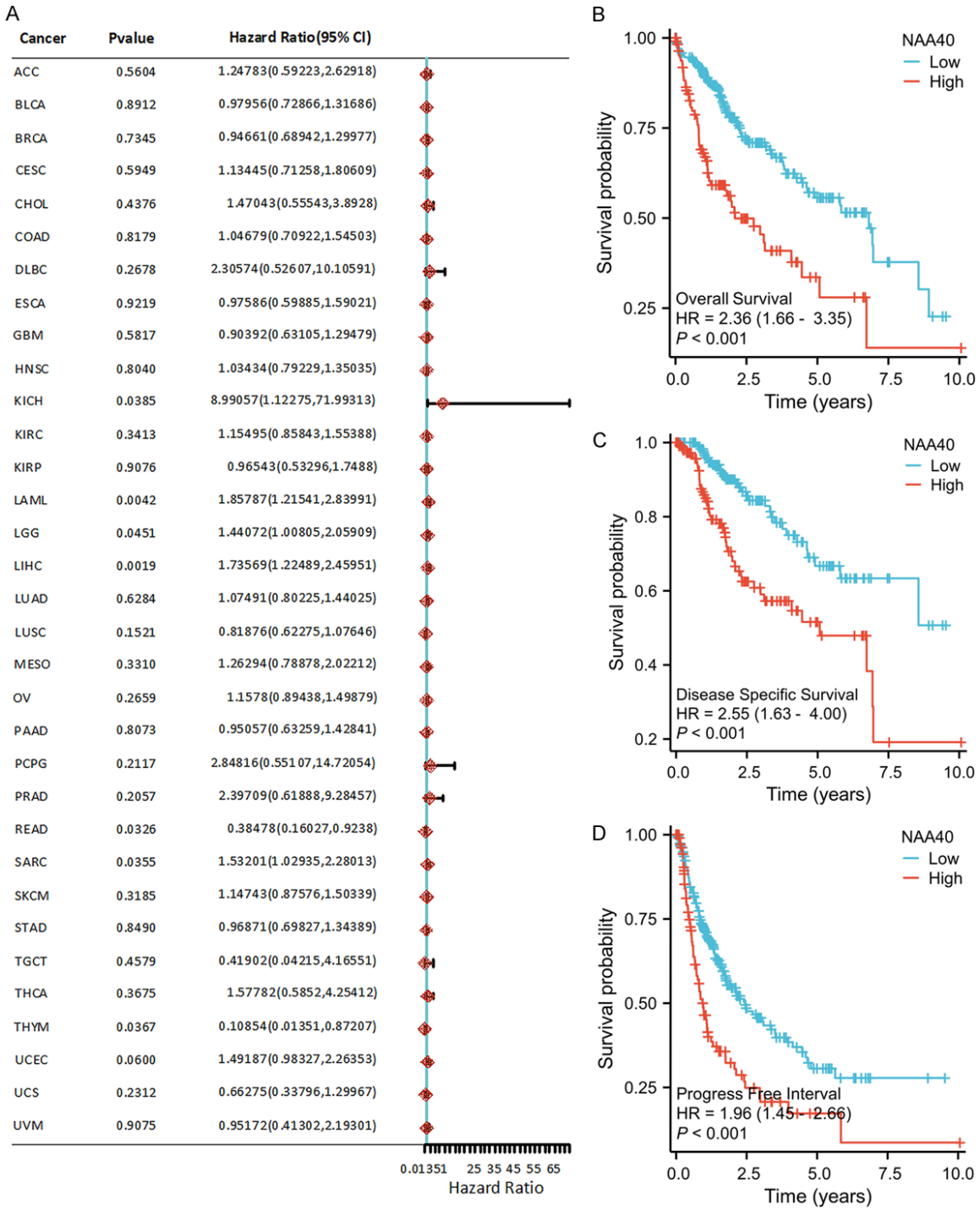


Figure 2. NAA40 expression correlated with prognosis. (A) The forest map based on multivariate Cox analysis for overall survival. The OS (B), DSS (C), and PFI (D) survival curves comparing patients with high (red) and low (blue) NAA40 expression in HCC were plotted at the threshold of P<0.05.

95% CI=1.66-3.35, P<0.001), disease-specific survival (DSS) (HR=2.55, 95% CI=1.63-4.00, P<0.001), and progression-free interval (PFI) (HR=1.96, 95% CI=1.45-2.66, P<0.001) (**Figure 2B-D**). Collectively, these findings underscore the substantial impact of NAA40 overexpression on prognosis, indicating a significantly worse clinical outcome.

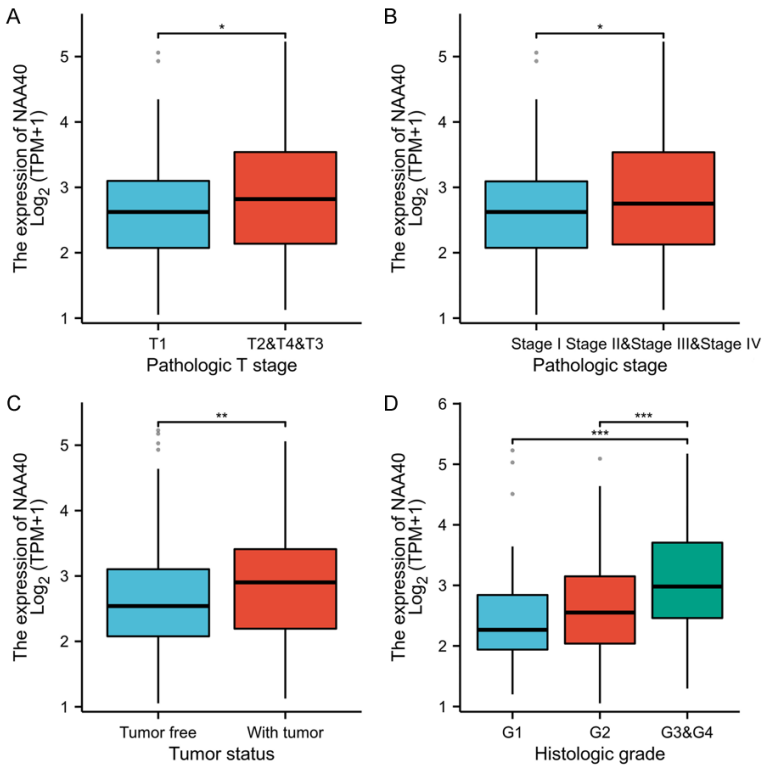


Figure 3. Relationship between the expression of NAA40 and clinicopathologic variables. The expression of NAA40 notably correlated with T stage (A), pathologic stage (B), tumor status (C), and histologic grade (D). *P<0.05, **P<0.01, and ***P<0.001.

Relationship between the expression of NAA40 and clinicopathologic variables

As depicted in **Figure 3**, the expression of NAA40 showed significant correlations with various clinical parameters. Notably, correlation was observed with T stage (T1 vs. T2 & T4 & T3, P<0.05), pathologic stage (stage I vs. stage II & stage III & stage IV, P<0.05), tumor status (tumor-free vs. with tumor, P<0.01), and histologic grade (G1 vs. G2 vs. G3 & G4, P<0.001).

Association between NAA40 expression with immune infiltration level

To examine the correlation between NAA40 expression and immune infiltration levels in HCC, we conducted ssGSEA analysis. The results revealed a distinct pattern: immune cells with high NAA40 expression exhibited a lower degree of immune infiltration compared to the low expression group. Notable differences were observed across various cell types, including aDC, cytotoxic cells, DC, eosinophils, iDC, mast cells, neutrophils, NK CD56dim cells, NK cells, pDC, T helper cells, Tcm, Tem, Tgd, Th17 cells, and Th2 cells (**Figure 4**).

Functional enrichment analysis

The volcano plot representing NAA40-related DEGs showcased significant down-regulation in blue and up-regulation in red, meeting the criteria of adjusted *p* value <0.05 and |log2-FC| >1. The results of DEGs are shown in Supplementary Table 1. Among the top up-regulated genes were TEX15, CDH10, MEGEA4, SLC6A15, and CEACAM7. Conversely, the leading down-regulated genes comprised CYP1A2, ANKFN1, SPINT3, SMR3A, and KLK3 (**Figure 5A**).

The GO enrichment analysis encompassed three key functional categories: biological processes, cellular components, and molecular functions. In terms of biological processes, significant terms included “nuclear division”, “xenobiotic metabolic process”, and “meiotic nuclear division”. Within molecular functions, prominent roles were associated with “iron ion binding”, “heme binding”, and “tetrapyrrole binding”. The cellular components category highlighted “apical plasma membrane”, “collagen-containing extracellular matrix”, and “basal plasma membrane”. Furthermore, the KEGG pathway analysis revealed notable enrichment of DEGs in pathways such as “metabolism of xenobiotics by cytochrome p450”, “retinol metabolism”, and “drug metabolism - cytochrome p450” (**Figure 5B**).

Subsequently, GSEA was utilized to compare the high and low NAA40 expression groups, uncovering significant enrichment of pathways including oxidative phosphorylation, xenobiotic metabolism, bile acid metabolism, fatty acid metabolism, G2M checkpoint, and E2F targets (**Figure 5C**).

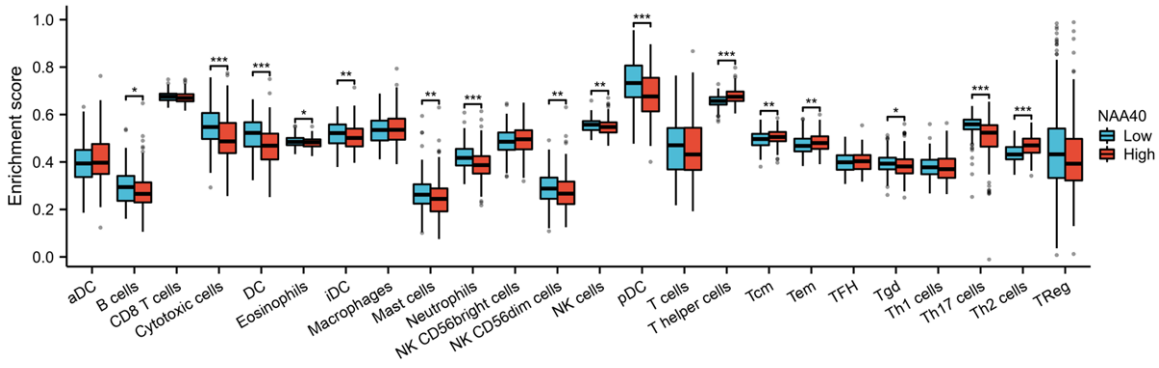
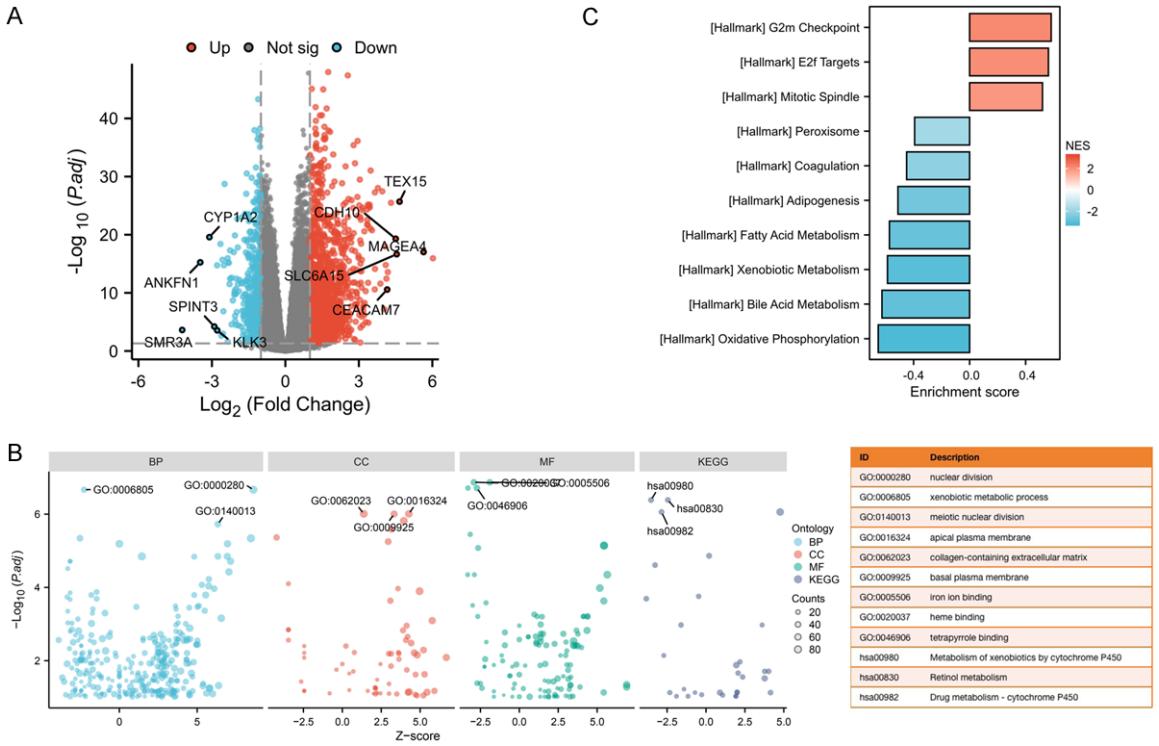


Figure 4. Association between NAA40 expression with immune infiltration level. *P<0.05, **P<0.01, and ***P<0.001.



ID	Description
GO:0000280	nuclear division
GO:0006805	xenobiotic metabolic process
GO:0140013	meiotic nuclear division
GO:0016324	apical plasma membrane
GO:0062023	collagen-containing extracellular matrix
GO:0009925	basal plasma membrane
GO:0005506	iron ion binding
GO:0020037	heme binding
GO:0046906	tetrapyrrole binding
hsa00980	Metabolism of xenobiotics by cytochrome P450
hsa00830	Retinol metabolism
hsa00982	Drug metabolism - cytochrome P450

Figure 5. Functional annotations and predicted signaling pathways. A. Volcano plot of DEGs. B. GO term and KEGG pathway enrichment analyses. C. GSEA Halmark pathway analysis.

Discussion

Currently, hepatocellular carcinoma (HCC) is marked by a dismal prognosis and elevated mortality rates, largely attributed to late-stage diagnosis, heightened metastasis rates, and rapid malignant progression [12]. Therefore, investigations into early diagnostic markers and effective therapeutic strategies hold significant potential to enhance the survival prospects of HCC patients. While NAA40 in the progression of numerous tumors is acknowledged, its precise influence on the prognosis and immune infiltration of HCC patients, along with the underlying mechanisms, remains largely unexplored.

In this study, we meticulously examined the expression level of NAA40 in HCC tissues, contrasting it with adjacent non-cancerous tissues, employing datasets from the TCGA and GETx databases. Notably, our findings underscored

higher NAA40 expression levels within HCC tissues, further strengthening the notion of its potential role as a significant biomarker. Furthermore, our data illuminated a compelling association between elevated NAA40 expression and adverse outcomes in HCC patients, encompassing overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI). This pivotal observation suggests that NAA40 plays a contributory role in HCC disease progression, serving as a prominent biomarker. These findings resonate with those reported in colorectal cancer [13] and breast cancer [14].

Additionally, our investigation revealed a significant correlation between NAA40 expression and critical clinical parameters, including T stage (T2 & T4 & T3), pathologic stage (stage II & stage III & stage IV), and histologic grade (G3 & G4). Previous studies have confirmed that high NAA40 expression is overexpressed in liver cancer [8], colorectal cancer [13, 15], lung cancer [5] and breast cancer [14]. However, there is still a lack of sufficient literature to explain the clinicopathological and prognostic significance of NAA40 overexpression in HCC. Our conclusion on the increased expression of NAA40 in liver cancer tissues based on the database was consistent with the results of Costas Koufaris et al. [9], but different from the results of Liu et al. [8], who emphasized that NAA40 was down-regulated in liver cancer tissues. Since the sample size of Liu's study was smaller, and the sample size of our experiment was more sufficient, we can believe that the conclusion of the upregulation of NAA40 expression in HCC is more reliable in the results of this study.

Immunotherapy, positioned as the fourth treatment modality alongside surgery, radiation, and chemotherapy, has garnered significant attention and is rapidly advancing as a frontline approach in diverse cancer treatments. The liver serves as a pivotal organ, initiating inflammatory responses that stimulate the body's immune reaction to counteract unwelcome pathogenic threats and the initiation of tumors [16]. However, the evasion of immune surveillance often stands as a pivotal characteristic driving the progression of hepatocellular carcinoma (HCC) [17]. Consequently, there is an imperative to unravel the intricate immune processes within HCC, with the goal of effectively harnessing anti-tumor immunity. Immunotherapeutic strategies for HCC can be broadly classified into immune checkpoint blockade (ICB), cell-based (primarily DC)/non-cell-based vaccines, adoptive cell transfer (ACT), cytokine/antibody immunization protocols, and combinations of immunotherapy agents with other drugs. The exploration of immune cell infiltration states in HCC not only holds the potential to sharpen the focus of immunotherapy but also to provide predictive insights into HCC outcomes. Tumors flourish within the tumor microenvironment (TME), a milieu that extends beyond tumor cells to encompass innate and adaptive immune cells, stromal components, endothelial cells, and cancer-associated fibroblasts [18]. This TME significantly influences the proliferation, migration, and angiogenesis of tumor cells [19].

Our findings highlight a negative correlation between NAA40 overexpression and the infiltration of immune cells, including aDC, cytotoxic cells, DC, eosinophils, iDC, mast cells, neutrophils, NK CD56dim cells, NK cells, pDC, T helper cells, Tcm, Tem, Tgd, Th17 cells, and Th2 cells. Notably, DC cells within the liver play a pivotal role in antigen uptake, migrating to regional lymph nodes where they mature and present antigens to non-allergic T cells. This dynamic process assumes a critical role in tumorigenesis and viral infections [20]. Similarly, NK cells in the liver exhibit robust activity and function as the frontline immune responders against tumor cells. However, their efficacy is compromised in various cancers, including HCC [21]. Reduced immune cell infiltration or suppressed immune cell function can lead to compromised human immune responses, thus promoting HCC development. Collectively, these findings suggest that NAA40 may potentially emerge as a novel target molecule implicated in HCC development, exerting influence over the TME and establishing associations with diverse immune cell infiltrations.

To delve deeper into the mechanistic underpinnings of NAA40's functional effects in HCC, we conducted an analysis of genes significantly associated with NAA40. This analysis encompassed both up-regulated and down-regulated genes exhibiting substantial abnormal expression. Employing GSEA enrichment, we unveiled

that these co-expressed genes alongside NAA40 could expedite the progression of HCC by facilitating biological processes such as nuclear division and cell metabolism. Furthermore, ample studies have underscored the involvement of the CYP450 family members in tumor diagnosis and treatment. For instance, downregulation of CYP39A1 has been correlated with adverse HCC carcinogenesis, tumor differentiation, and overall survival [22]. Additionally, CYP3A5 has demonstrated tumor-suppressive attributes by regulating the mTORC2/Akt signaling pathway in HCC [23]. Our KEGG analysis hinted at NAA40's potential to inhibit cytochrome P450 enzymes present in the liver, thus impeding the metabolic transformation of numerous biological compounds and potentially exacerbating HCC progression.

Additionally, we dissected the differences in GSEA outcomes within Hallmark pathways between high NAA40 gene expression and low NAA40 gene expression. These findings revealed a noteworthy up-regulation in G2M checkpoint and E2F targets enrichment in the high NAA40 gene expression group. Conversely, oxidative phosphorylation, xenobiotic metabolism, bile acid metabolism, and fatty acid metabolism exhibited down-regulation. These findings necessitate further experimental validation to substantiate the biological function and role of NAA40 in HCC.

Despite providing fresh insights into the link between NAA40 expression and its prognostic significance in HCC, our study has certain limitations that warrant consideration and further exploration. First and foremost, the precise mechanism through which NAA40 influences disease progression via immune infiltration requires in-depth exploration, necessitating rigorous in vitro and in vivo investigations to unveil the underlying biological functions and potential mechanisms. Second, a more extensive range of clinical trials and data is imperative to unravel the molecular mechanisms specific to HCC. Third, our findings should ideally undergo validation via comprehensive genomics studies.

In summary, our study verifies the distinct expression patterns of NAA40 in cancerous and adjacent tissues, assesses the prognostic implications for both NAA40 and HCC patients, and initiates a preliminary exploration of molecular functions and associated mechanisms at the level of immune infiltration of NAA40 in HCC. These outcomes corroborate the role of NAA40 in HCC development, potentially enriching our understanding of its pertinent biological functions. Furthermore, they propose NAA40 as a novel prognostic biomarker target for the formulation of anti-cancer strategies tailored to HCC.

Disclosure of conflict of interest

None.

Abbreviations

BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; HNSC, head & neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; NATs, N-terminal acetyltransferase; HCC, hepatocellular carcinoma; EMT, epithelial-to-mesenchymal transition; TCGA, the Cancer Genome Atlas; GTEx, Genotype Tissue Expression Project; DEGs, differential expression of genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene set enrichment analysis; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; ICB, immune checkpoint blockade; ACT, adoptive cell transfer; TME, tumor microenvironment.

Address correspondence to: Bin Feng, Center for Clinical Laboratory, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China. E-mail: 18962111558@189.cn

References

[1] Siegel RL, Miller KD, Wagle NS and Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023; 73: 17-48.

[2] Ganesan P and Kulik LM. Hepatocellular carcinoma: new developments. Clin Liver Dis 2023; 27: 85-102.

[3] Zhang G, Li R, Deng Y and Zhao L. Conditional survival of patients with hepatocellular carcinoma: results from the surveillance, epidemiology, and end results registry. Expert Rev Gastroenterol Hepatol 2018; 12: 515-523.

[4] Molina-Serrano D, Schiza V, Demosthenous C, Stavrou E, Oppelt J, Kyriakou D, Liu W, Zisser G,

Bergler H, Dang W and Kirmizis A. Loss of Nat4 and its associated histone H4 N-terminal acetylation mediates calorie restriction-induced longevity. EMBO Rep 2016; 17: 1829-1843.

[5] Deng Y, Deng S, Ho YH, Gardner SM, Huang Z, Marmorstein R and Huang R. Novel bisubstrate inhibitors for protein N-terminal acetyltransferase D. J Med Chem 2021; 64: 8263-8271.

[6] Ju J, Chen A, Deng Y, Liu M, Wang Y, Wang Y, Nie M, Wang C, Ding H, Yao B, Gui T, Li X, Xu Z, Ma C, Song Y, Kvansakul M, Zen K, Zhang CY, Luo C, Fang M, Huang DCS, Allis CD, Tan R, Zeng CK, Wei J and Zhao Q. NatD promotes lung cancer progression by preventing histone H4 serine phosphorylation to activate slug expression. Nat Commun 2017; 8: 928.

[7] Koufaris C and Kirmizis A. N-terminal acetyltransferases are cancer-essential genes prevalently upregulated in tumours. Cancers (Basel) 2020; 12: 2631.

[8] Liu Z, Liu Y, Wang H, Ge X, Jin Q, Ding G, Hu Y, Zhou B, Chen Z, Ge X, Zhang B, Man X and Zhai Q. Patt1, a novel protein acetyltransferase that is highly expressed in liver and downregulated in hepatocellular carcinoma, enhances apoptosis of hepatoma cells. Int J Biochem Cell Biol 2009; 41: 2528-2537.

[9] Koufaris C and Kirmizis A. Identification of NAA40 as a potential prognostic marker for aggressive liver cancer subtypes. Front Oncol 2021; 11: 691950.

[10] Hänzelmann S, Castelo R and Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 2013; 14: 7.

[11] Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, Bruneval P, Fridman WH, Becker C, Pagès F, Speicher MR, Trajanoski Z and Galon J. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013; 39: 782-795.

[12] Gilles H, Garbutt T and Landrum J. Hepatocellular carcinoma. Crit Care Nurs Clin North Am 2022; 34: 289-301.

[13] Demetriadou C, Pavlou D, Mpekris F, Achilleos C, Stylianopoulos T, Zaravinos A, Papageorgis P and Kirmizis A. NAA40 contributes to colorectal cancer growth by controlling PRMT5 expression. Cell Death Dis 2019; 10: 236.

[14] Lee KM, Park SK, Kim SU, Doll MA, Yoo KY, Ahn SH, Noh DY, Hirvonen A, Hein DW and Kang D. N-acetyltransferase (NAT1, NAT2) and glutathione S-transferase (GSTM1, GSTT1) polymorphisms in breast cancer. Cancer Lett 2003; 196: 179-186.

[15] Pavlou D and Kirmizis A. Depletion of histone N-terminal-acetyltransferase Naa40 induces p53-independent apoptosis in colorectal cancer cells via the mitochondrial pathway. Apoptosis 2016; 21: 298-311.

[16] Fu Y, Liu S, Zeng S and Shen H. From bench to bed: the tumor immune microenvironment and current immunotherapeutic strategies for hepatocellular carcinoma. J Exp Clin Cancer Res 2019; 38: 396.

[17] Shlomai A, de Jong YP and Rice CM. Virus associated malignancies: the role of viral hepatitis in hepatocellular carcinoma. Semin Cancer Biol 2014; 26: 78-88.

[18] Oura K, Morishita A, Tani J and Masaki T. Tumor immune microenvironment and immunosuppressive therapy in hepatocellular carcinoma: a review. Int J Mol Sci 2021; 22: 5801.

[19] Hinshaw DC and Shevde LA. The tumor microenvironment innately modulates cancer progression. Cancer Res 2019; 79: 4557-4566.

[20] Lurje I, Hammerich L and Tacke F. Dendritic cell and T cell crosstalk in liver fibrogenesis and hepatocarcinogenesis: implications for prevention and therapy of liver cancer. Int J Mol Sci 2020; 21: 7378.

[21] Liu P, Chen L and Zhang H. Natural killer cells in liver disease and hepatocellular carcinoma and the NK cell-based immunotherapy. J Immunol Res 2018; 2018: 1206737.

[22] Li D, Yu T, Hu J, Wu J, Feng S, Xu Q, Zhu H, Zhang X, Zhang Y, Zhou B, Gu L and Zeng Z. Downregulation of CYP39A1 serves as a novel biomarker in hepatocellular carcinoma with worse clinical outcome. Oxid Med Cell Longev 2021; 2021: 5175581.

[23] Jiang F, Chen L, Yang YC, Wang XM, Wang RY, Li L, Wen W, Chang YX, Chen CY, Tang J, Liu GM, Huang WT, Xu L and Wang HY. CYP3A5 functions as a tumor suppressor in hepatocellular carcinoma by regulating mTORC2/Akt signaling. Cancer Res 2015; 75: 1470-1481.