Original Article

NOVA2 regulates the properties of liver cancer stem cells and lenvatinib resistance in hepatocellular carcinoma via the Wnt pathway

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Background: The regulation of cancer stem cells (CSCs) is influenced by RNA-binding proteins (RBPs). The present study sought to investigate the role of NOVA2 in the processes of self-renewal, carcinogenesis, and lenvatinib resistance in liver CSCs.

Methods: Neuro-oncological ventral antigen 2 (NOVA2) expression in liver CSCs was examined by real-time polymerase chain reaction (PCR). *In vitro* experiments were used to assess the effects of NOVA2 on liver CSC expansion and lenvatinib resistance.

Results: In our study, the expression of the RBP NOVA2 was higher in CSCs. NOVA2 also increased the capacity for self-renewal and carcinogenesis of the liver CSCs via the Wnt pathway. Further, suppressing the Wnt pathway leads to desensitization of the hepatocellular carcinoma (HCC) cells that overexpress NOVA2 to apoptosis caused by lenvatinib. Analyzing patient data confirmed reduced levels of NOVA2 and therefore we speculate that NOVA2 may serve as a potential indicator for response to lenvatinib in patients with HCC. Methyltransferase-like 3 (METTL3) and YTH N6-methyladenosine RNA-binding protein 1 (YTHDF1)-dependent N6-methyladenosine (m^6A) methylation were linked to upregulation of NOVA2 in HCC. Furthermore, it was shown that the expression of METTL3 was elevated in cellular models of type 2 diabetes mellitus (T2DM).

Conclusions: NOVA2 is involved in the process of liver CSC self-renewal and carcinogenesis. In addition, NOVA2 expression may help identify patients with a higher chance of benefiting from lenvatinib treatment and can be a promising therapeutic target for HCC.

Keywords: Liver cancer stem cell (liver CSC); neuro-oncological ventral antigen 2 (NOVA2); hepatocellular carcinoma (HCC); N6-methyladenosine (m^6A); lenvatinib

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Introduction

Hepatocellular carcinoma (HCC) continues to be a significant global concern in the context of liver disease (1-3). Lenvatinib was recently approved as a targeted therapeutic agent for the management of advanced HCC (4). However, previous studies have shown that some individuals with HCC have limited sensitivity to lenvatinib (5,6). Thus, investigating resistance mechanisms is crucial.

Cancer stem cells (CSCs), also known as tumor-initiation cells (T-ICs), are a subpopulation of cancer cells with the ability to proliferate indefinitely and undergo self-renewal (7,8). A considerable body of evidence suggests that CSCs regulate several biological processes associated with cancers, such as recurrence, proliferation, initiation, metastasis, and the development of treatment resistance (9-11). Previous studies identified CSCs as the primary determinant of tumor metastasis and recurrence (12-14). To date, the precise mechanism by which liver CSCs are involved in carcinogenesis remains unclear.

A study has shown that RNA-binding proteins (RBPs) that regulate RNA modification and quality are important factors in the progression and development of cancer (15). Neuro-oncological ventral antigen 2 (NOVA2), an RBP, is a constituent of the NOVA gene family and plays a significant role in the viability and maturation of motoneurons (or motor neurons) (16). According to a previous research, NOVA2 is involved in the development of several types of cancer (17), the precise function of NOVA2 in liver CSCs remains unknown. The present study sought to investigate the role of NOVA2 in the processes of self-renewal, carcinogenesis, and lenvatinib resistance in liver CSCs. We present this article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-24-145/rc).

Highlight box

Key findings

- Our study shows that neuro-oncological ventral antigen 2 (NOVA2) is involved in the process of liver cancer stem cell (CSC) self-renewal and carcinogenesis.

What is known and what is new?

- NOVA2 contributes to the development of several types of cancer.
- This study confirmed that NOVA2 is significantly associated with the self-renewal and carcinogenesis of liver CSCs, and NOVA2 may be a potential therapeutic option for hepatocellular carcinoma (HCC).

What is the implication, and what should change now?

- This study shows that NOVA2 is significantly involved in the process of liver CSC self-renewal and carcinogenesis. Thus, NOVA2 may be a promising therapeutic target for HCC.

Methods

HCC samples

The study group comprised 30 patients (the histopathological types of the resected HCCs include 15 trabecular, six trabecular, five pseudoglandular duct, and four patchy types) who received adjuvant lenvatinib therapy (due to postoperative recurrence and inability to undergo surgical treatment, the patient received lenvatinib treatment) after surgical intervention for primary HCC (median survival time was 23.16 months, and the 5-year survival proportion was 44%) at the Eastern Hepatobiliary Surgery Hospital from 2012 to 2020. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all the patients, and the study was approved by the ethics committee of Eastern Hepatobiliary Surgery Hospital, Naval Medical University (No. EHBHKY2024-K014-P001).

Cell culture and treatments

The L02, Huh7, and Hep3B cell lines were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM of L-glutamine, and 25 μg/L of gentamicin. The cells were incubated at 37 ℃ in a 5% carbon dioxide incubator. The HCC cells were infected with lentivirus that had either NOVA2 knockdown or NOVA2 overexpression constructs, or a control lentivirus obtained from Ribobio (Shanghai, China). The resulting transfected cells that showed stability were screened using puromycin. The cells were cultivated

for 24 hours in DMEM supplemented with 5 mM of glucosamine (G1514, Sigma-Aldrich, Shanghai, China) to establish a cellular model of type 2 diabetes mellitus (T2DM).

Lenvatinib-resistant cells

Lenvatinib-resistant cell lines derived from HCC cells were incubated with lenvatinib (S1164, Selleck, Houston, TX, USA) at a concentration slightly lower than their half-maximal inhibitory concentration (0.25 μM/L). The concentration of lenvatinib was gradually increased by 0.2 μM/L per week over 9 months. Three cell lines that exhibited resistance to lenvatinib were acquired and then sustained by ongoing cultivation in the presence of lenvatinib.

Spheroid assays

The HCC cells were cultured in 96-well ultra-low attachment culture plates (Corning Incorporated Life Sciences, Corning, NY, USA) at a density of 300 cells per well (18). The number of spheroids was determined, and representative perspectives were provided.

In vitro limiting dilution assays

The HCC cells were cultivated in 96-well ultra-low attachment culture plates and seeded at different densities of 2, 4, 8, 16, 32, and 64 cells/well, with eight replicates. DMEM/Ham's F 12 nutrient medium (Gibco, Grand Island, NY, USA), supplemented with 1% FBS, 20 ng/mL of basic fibroblast growth factor, and 20 ng/mL of epidermal growth factor was used as the culture medium. The cells remained in this culture medium for 1 week, we used ELDA software (18) (http://bioinf.wehi.edu.au/software/elda/index.html) to evaluate the percentage of CSCs.

Flow cytometry

To identify CD133 and CD90 positive cells, we incubated cells from the HCC tumors and cells from patients' HCC with primary antibodies against CD133 (Biolegend, Inc., San Diego, CA, USA) or CD90 (Biolegend, Inc.) for 30 minutes at ambient temperature. The cells were subsequently analyzed with flow cytometry with a MoFlo XDP cell sorter (Beckman Coulter, Indianapolis, IN, USA), following the guidelines provided by the manufacturer.

Apoptosis assays

A 10 μM dose of lenvatinib was applied to the HCC cells for 48 hours. The cells were stained with Annexin V and 7-amino actinomycin D (Beyotime, Shanghai, China) for 15 minutes at room temperature and kept in the dark. The apoptotic cells were identified using the Annexin V-FITC Apoptosis Detection Kit I (BD Pharmingen, San Diego, CA, USA) and a flow cytometry, following the guidelines provided by the manufacturer.

RNA immunoprecipitation (RIP) assays

A Magna RIP RBP Immunoprecipitation Kit (17-701, Millipore, Bedford, MA, USA) was used for the RIP experiments as described previously (18).

Luciferase reporter assays

The luciferase reporter assays were performed following an established protocol (18).

Real-time polymerase chain reaction (PCR)

Total RNA was isolated from the cells using a methodology outlined in a previous study (18). Subsequently, the RNA was subjected to reverse transcription using the M-MLV RTase cDNA Synthesis Kit (Promega, Madison, WI, USA). The real-time PCR study was conducted using Roche's SYBR Green PCR Kit in conjunction with the LightCycler 480 System (Roche, Basel, Switzerland). The PCR primers were obtained from Shanghai Yuanzhi Biotechnology Co., Ltd. (Shanghai, China).

Western blot assays

The assays were performed using a previously reported method (18). A LI-COR imaging system (LI-COR Biosciences, Shanghai, China) was used to detect the fluorescence intensity.

Statistical analysis

We present our data with mean ± standard deviation (SD). For the statistical analyses we used GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). A P value of less than 0.05 was considered statistically significant.

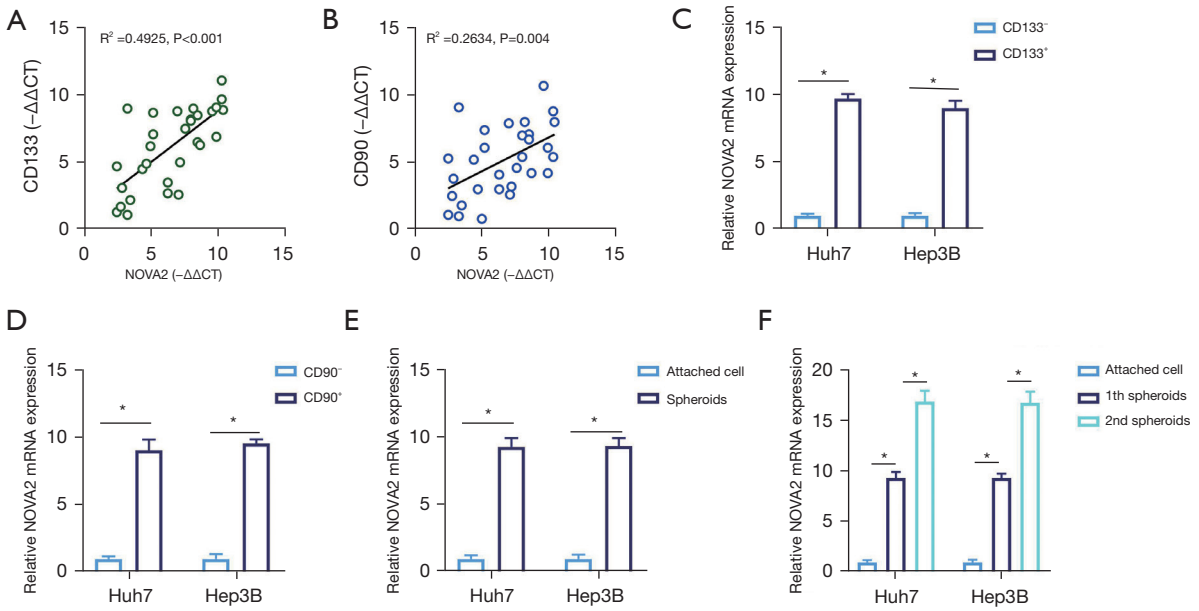

Figure 1 The upregulated expression of NOVA2 in liver CSCs. (A,B) The correlation between the level of NOVA2, CD133, and CD90 in the primary HCC cells was examined (n=30). (C) NOVA2 expression in the CD133$^{+/-}$ HCC cells was analyzed. (D) NOVA2 expression in the CD90$^{+/-}$ HCC cells was analyzed. (E) NOVA2 expression in the HCC spheroids and attached cells was determined. (F) NOVA2 expression in the serial passages of the HCC spheroids was determined. *, P<0.05. CT, cycle threshold; NOVA2, neuro-oncological ventral antigen 2; mRNA, messenger RNA; CSC, cancer stem cell; HCC, hepatocellular carcinoma.

Results

NOVA2 is upregulated in liver CSCs

Figure 1A,1B shows a strong correlation between the levels of NOVA2 and the expression of CD133 and CD90; both are well-recognized markers for liver CSCs (19). These findings were observed in the tumor cells isolated from the primary HCC tissues. Expression of NOVA2 was significantly increased in CD133^{+} and CD90^{+} HCC cells compared to CD133^{-} or CD90^{-} HCC cells (*Figure 1C,1D*). Further, the expression of NOVA2 was higher in the HCC spheres than in the adherent HCC cells (*Figure 1E*). The level of NOVA2 increased progressively in successive passages of HCC spheroids (*Figure 1F*).

NOVA2 enhanced liver CSCs and self-renewal

To evaluate the possible role of NOVA2 in liver CSCs, the HCC cells were infected with a NOVA2 knockdown virus (*Figure 2A*). *Figure 2B,2C* displays that the cells with inhibited expression of NOVA2 show a reduced expression of the CSC markers. The self-renewal capacity was restrained, the proportion of CSCs was decreased in the NOVA2 knockdown cells (*Figure 2D,2E*). Conversely, the CSC markers were increased, the self-renewal capacity was intensified, the proportion of CSCs was increased in the NOVA2 overexpressed cells (*Figure 3A-3E*).

NOVA2 increased liver CSC self-renewal by activating the Wnt pathway

The Wnt/β-catenin pathway is a common process in CSCs (20). This study examined the potential of NOVA2 to promote liver CSCs by activating the Wnt pathway. The TOP/FOP-flash luciferase experiment showed that the inhibition of NOVA2 resulted in suppressed Wnt signaling activation (*Figure 4A*). Additionally, the knockdown of NOVA2 decreased AXIN2, c-MYC, and cyclin D1 levels (*Figure 4B*). The administration of adavivint, a Wnt inhibitor, also resulted in a reduction of the observed enhanced self-renewal capability in HCC cells overexpressing NOVA2. Further, the proportion of CSCs were elevated in the NOVA2 overexpressing cells compared to the control cells (*Figure 4C,4D*).

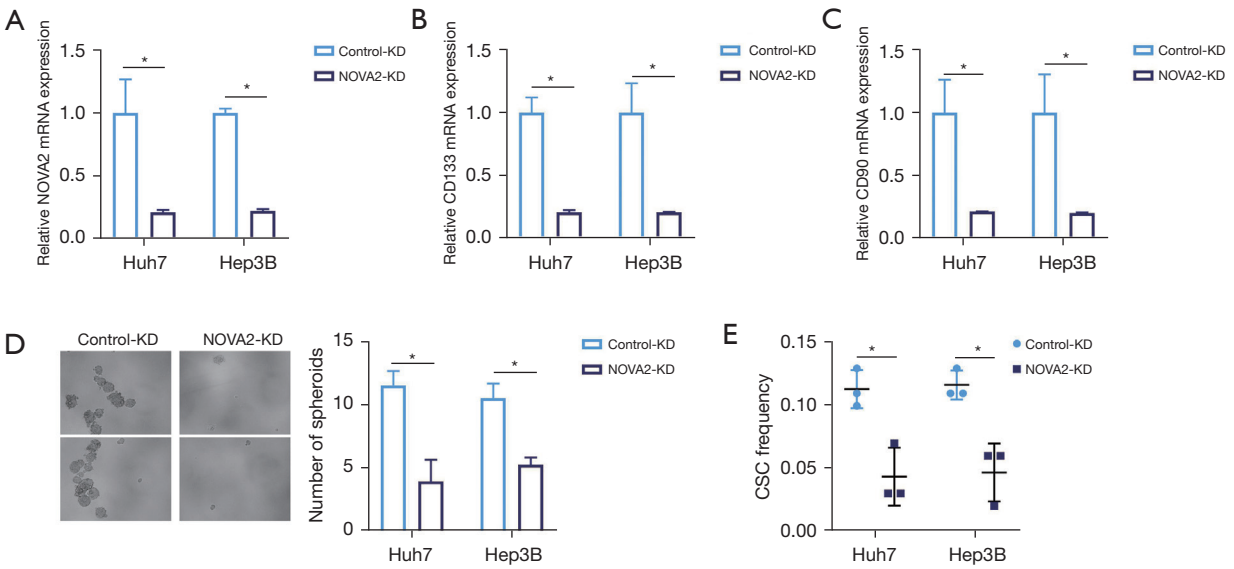

Figure 2 The knockdown of NOVA2 inhibits the self-renewal of liver CSCs. (A) The knockdown effect of NOVA2 was verified. (B,C) The CD133 and CD90 expression levels in the HCC cells was examined. (D) Assays for the spheroid formation of the specified HCC cells; representative images shown, magnification ×200. (E) An *in vitro* limiting dilution test was performed to show the proportion of liver CSCs in specified HCC cells. *, P<0.05. NOVA2, neuro-oncological ventral antigen 2; mRNA, messenger RNA; KD, knock down; CSC, cancer stem cell; HCC, hepatocellular carcinoma.

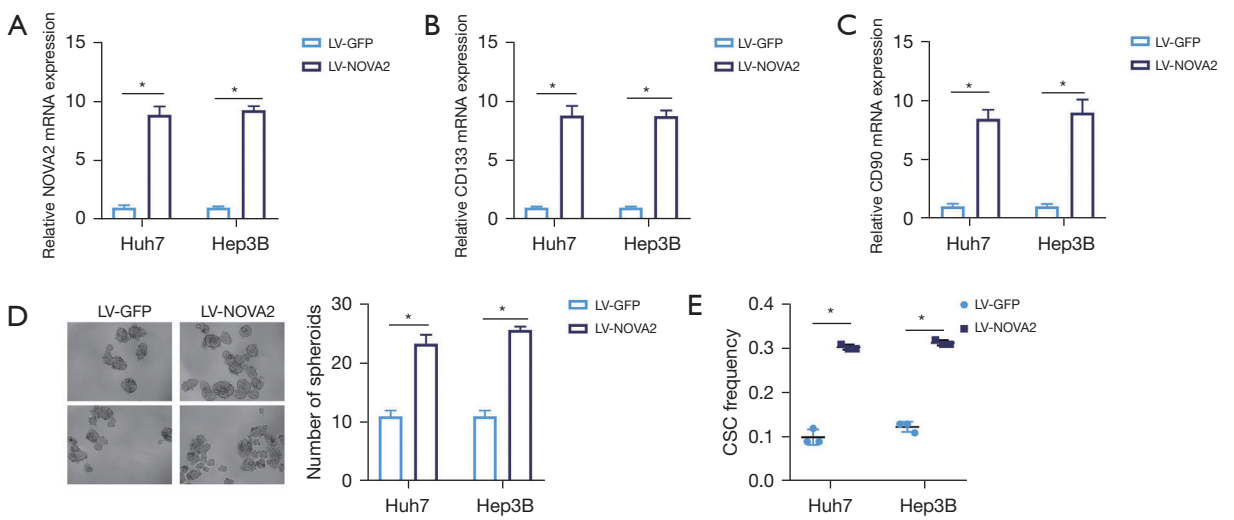

Figure 3 The overexpression of NOVA2 stimulates the self-renewal of liver CSCs. (A) NOVA2 overexpression was verified. (B,C) CD133 and CD90 in the indicated HCC cells. (D) Assays for the spheroid formation of the specified HCC cells; representative images shown, magnification ×200. (E) An *in vitro* limiting dilution test was performed to show the proportion of liver CSCs in specified HCC cells. *, P<0.05. NOVA2, neuro-oncological ventral antigen 2; mRNA, messenger RNA; LV, lentivirus; GFP, green fluorescent protein; CSC, cancer stem cell; HCC, hepatocellular carcinoma.

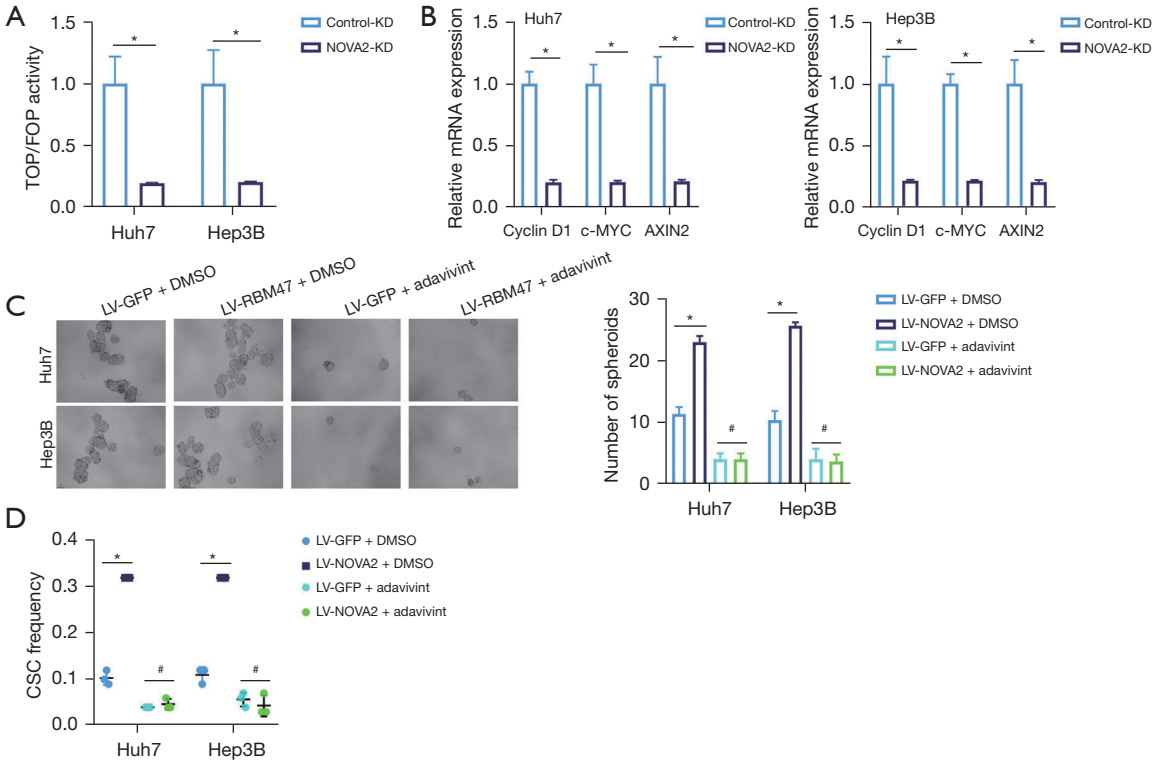

Figure 4 By stimulating the Wnt pathway, NOVA2 increased the self-renewal of liver CSCs. (A) A TOP/FOP-flash reporter analysis was conducted using specified HCC cells. (B) AXIN2, c-MYC, and cyclin D1 real-time PCR tests were performed in the specified HCC cells. (C) DMSO or adavivint cells were used for the spheroid generation experiment to infect the LV-GFP and LV-NOVA2 HCC cells; representative images are shown, magnification ×200. (D) An *in vitro* limiting dilution test was performed to show the proportion of liver CSCs in the LV-GFP and LV-NOVA2 HCC cells infected with DMSO or adavivint. *, P<0.05; #, P>0.05. NOVA2, neuro-oncological ventral antigen 2; KD, knock down; mRNA, messenger RNA; LV, lentivirus; GFP, green fluorescent protein; DMSO, dimethyl sulfoxide; CSC, cancer stem cell; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction.

NOVA2 determines lenvatinib response in HCC cells

CSCs play a crucial role in the modulation of chemoresistance (21). NOVA2 was significantly elevated in the HCC cell lines that developed a resistance to lenvatinib (*Figure 5A*). Additionally, as *Figure 5B* shows, the administration of adavivint counteracted the reduced sensitivity of HCC cells overexpressing NOVA2 to lenvatinib-induced cell apoptosis. Notably, Adjuvant treatment with lenvatinib in HCC patients with low NOVA2 expression achieved a better prognosis than patients with high NOVA2 expression (*Figure 5C*).

N6-methyladenosine (m^6A) RNA methylation mediates NOVA2 increases in HCC

The underlying mechanism by which NOVA2 is upregulated in HCC was explored. As *Figure 6A* shows, NOVA2 expression was not affected by histone acetylation or DNA methylation. Further, the knockdown of Dicer did not result in any significant alteration in NOVA2 expression in HCC (*Figure 6B*). Subsequently, the role of RNA methylation was explored. Specifically, the downregulation of m^6A-methylases, such as the methyltransferase-like 3 (METTL3), but not other methylases, such as METTL14 or Wilms' tumor 1-associating protein (WTAP), significantly reduced the expression of NOVA2. On the other hand, downregulation of m^6A-demethylases, such as the fat mass and obesity-associated (FTO) protein or AlkB homolog 5 (ALKBH5) protein, did not significantly affect the expression of NOVA2. Using the SRAMP website (http://www.cuilab.cn/sramp/), a total of eight m^6A-methylation locations were identified in the messenger RNA (mRNA) sequence of NOVA2 (*Figure 6C,6D*). METTL3 caused an

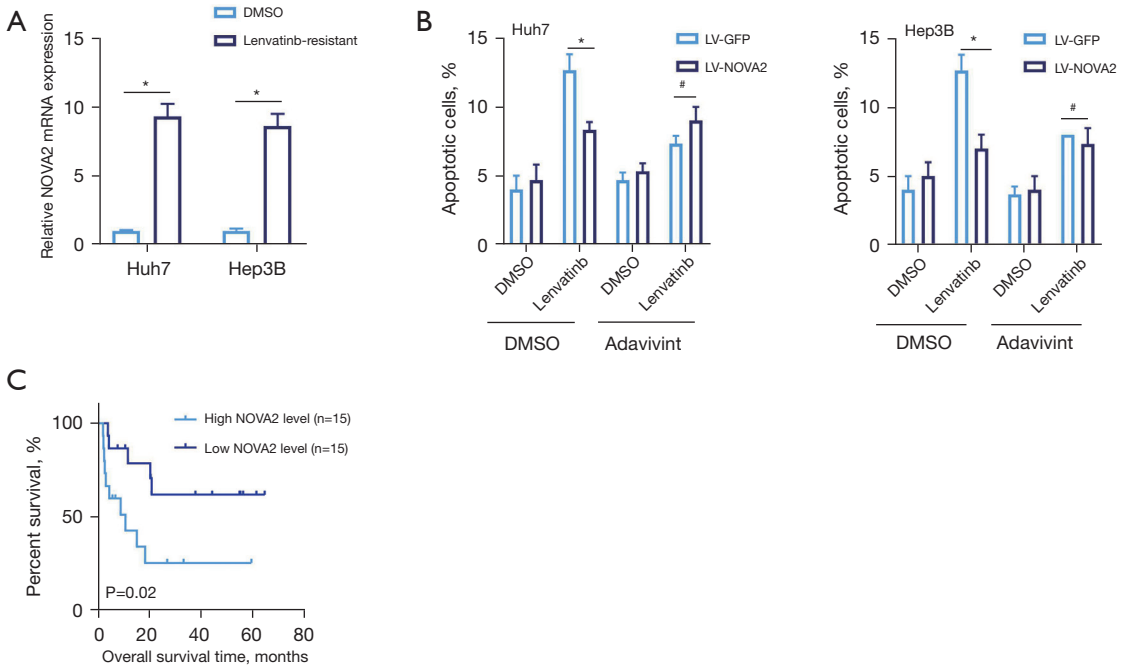

Figure 5 The response of lenvatinib in HCC cells was determined by NOVA2. (A) The expression of NOVA2 in the control HCC cells and the cells resistant to lenvatinib was examined. (B) LV-GFP and LV-NOVA2 HCC cells were treated with lenvatinib (10 μM) for 48 hours after being infected with DMSO or adavivint (10 μM). Using flow cytometry, the fraction of apoptotic cells was investigated. (C) In HCC patients treated with lenvatinib, the overall survival of patients between the NOVA2-high (n=15) or -low (n=15) groups were assessed by a Kaplan-Meier analysis. The high- and low- group of NOVA2 was divided by the mean level of NOVA2 in 30 HCC tissues. *, P<0.05; #, P>0.05. NOVA2, neuro-oncological ventral antigen 2; mRNA, messenger RNA; DMSO, dimethyl sulfoxide; LV, lentivirus; GFP, green fluorescent protein; HCC, hepatocellular carcinoma.

increase in the hyper-methylation of NOVA2 m^{6}A-peak-6 and -7 in the HCC cell lines (*Figure 6E*). MRNA stability is regulated by "reader proteins", which are influenced by RNA m^{6}A modification (21). The downregulation of YTH N6-methyladenosine RNA-binding protein 1 (YTHDF1), rather than insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), IGF2BP2, IGF2BP3, YTHDF2, or YTHDF3, led to a significant decrease in NOVA2 expression (*Figure 6F,6G*). In addition, the RIP investigation suggested an interaction between YTHDF1 and NOVA2 mRNA in HCC cells (*Figure 6H*). Further, in the HCC cells with YTHDF1 knockdown, the half-life of the NOVA2 transcripts was shortened (*Figure 6I*).

METTL3 expression was increased in patients with T2DM and the T2DM cell models

Insulin resistance is considered the most prominent clinical-pathological characteristic of T2DM, and it can be triggered by prolonged exposure to elevated amounts of glucose or glucosamine. Human hepatic carcinoma cells (Huh7) and non-tumor liver cells (L02) were used to establish a cellular model of T2DM. The cells were exposed to elevated glucose levels for 24 hours, and glucose uptake and glucose consumption experiments were then performed. The capacity of the cells to absorb glucose was significantly reduced when the cells were incubated in a medium with consistently high glucose levels, as shown by the decreased glucose consumption in response to insulin (*Figure 7A,7B*). Measurements were made to ascertain the expression levels of METTL3 in the two cell types linked to T2DM to explore the function of this protein in this specific process. The analysis showed that METTL3 expression was significantly increased in both models (*Figure 7C*). Conversely, the two cell lines were subjected to glucosamine, a known inducer of insulin resistance, for 24 hours, and the upregulation of METTL3 was observed (*Figure 7D*). Thus, the overexpression of METTL3,

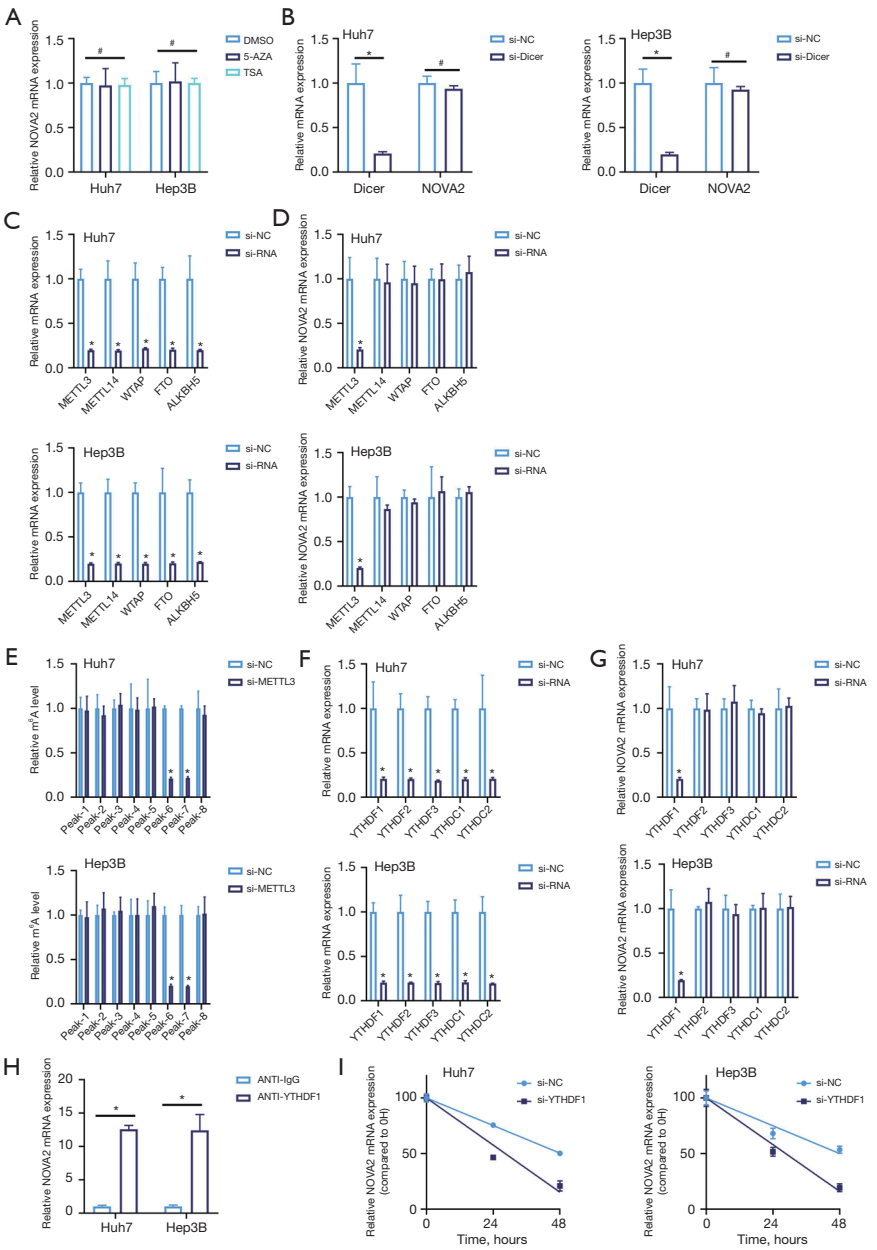

Figure 6 Methylation of m^{6}A RNA mediates the upregulation of NOVA2 in HCC. (A) Following treatment with DMSO, 5-AZA, or TSA, the NOVA2 levels of the HCC cells were assessed. (B) The NOVA2 levels of the HCC cells that had been transfected with si-Dicer or si-NC were analyzed. (C) A quantitative PCR analysis was performed on the HCC cells transfected with the specified short-interfering RNAs (si-METTL3/METTL14/WTAP/FTO/ALKBH5 or si-NC). (D) The NOVA2 levels were examined by quantitative RT-PCR in the identified HCC cell lines. (E) m^{6}A-quantitative PCR was used to quantify m6-site levels of NOVA2 in the specified HCC cells. (F) A quantitative PCR analysis was performed on the HCC cells transfected with the specified short-interfering RNAs (si-YTHDC-1/-2, si-YTHDF-1/-2/-3, or si-NC). (G) The levels of NOVA2 were examined in the HCC cells transfected with the specified short-interfering RNAs (si-NC, si-YTHDF-1/-2-3, or si-YTHDC-1/-2). (H) NOVA2 binding to the YTHDF1 protein in HCC was verified by RIP-quantitative PCR. (I) Half-life of NOVA2 transcripts in indicated HCC cells. *, P<0.05; #, P>0.05. NOVA2, neuro-oncological ventral antigen 2; mRNA, messenger RNA; DMSO, dimethyl sulfoxide; 5-AZA, 5-azacytidine; TSA, trichostatin A; si, small interference; NC, negative control; m^{6}A, N6-methyladenosine; IgG, immunoglobulin G; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction; RT, reverse transcription; RIP, RNA immunoprecipitation.

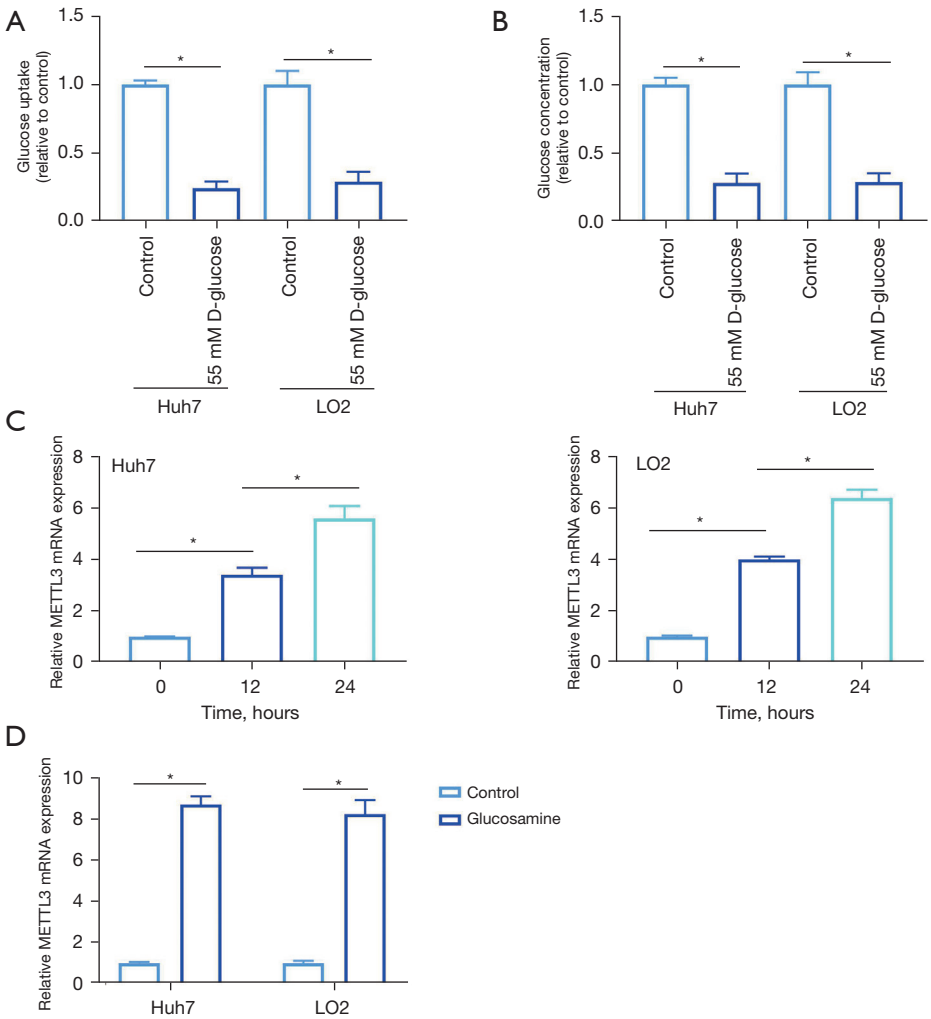

Figure 7 METTL3 was upregulated in T2DM cell models. (A) Cell glucose uptake was examined before and after high glucose therapy. (B) Normal glucose intake was examined in cells pre-treated with high or normal glucose DMEM for 6 hours. (C) METTL3 was detected in cells treated with high glucose at 0, 12, and 24 hours. (D) 24-hour METTL3 expression was examined in cells treated with 5 mM of glucosamine. *, P<0.05. mRNA, messenger RNA; T2DM, type 2 diabetes; DMEM, Dulbecco's modified Eagle's medium.

caused by prolonged exposure to high amounts of glucose or glucosamine, is linked to the suppression of glucose absorption in cell models of T2DM.

Discussion

Recently, a growing body of data has indicated that RBPs play crucial roles in regulating CSCs or T-ICs (22-25). A previous study has shown that NOVA2 is involved in the pathogenesis of many types of cancer (17). However, the precise function of NOVA2 in liver CSCs remained unclear. The findings of our study shed light on the pivotal role of NOVA2 in regulating the properties of CSCs and influencing resistance to lenvatinib in HCC. This significant insight into the molecular mechanisms underlying HCC treatment opens new avenues for therapeutic interventions. Identifying NOVA2 as a critical player in lenvatinib resistance suggests that combination therapies involving NOVA2 inhibition could enhance the efficacy of existing treatment strategies.

m^6A is a common internal alteration in mRNA molecules, particularly in mammalian cells, and it mostly occurs in the last exon of mRNA sequences (26). The methylation process is regulated by proteins known as "writer", "eraser",

and "reader" proteins (27). The m^{6}A methyltransferase complex primarily comprises METTL3, METTL14, and WTAP, which function as "writers" that are responsible for facilitating methylation reactions. Conversely, FTO and ALKBH5 serve as demethylases, and play the role of "erasers". The m^{6}A methylated mRNA is recognition by certain "reader" proteins, such as YTHDF1, YTHDF2, and YTHDF3. This recognition process has implications for several aspects of mRNA function, including stability, translation, nuclear transportation, and splicing (28).

Several m^{6}A targets have been implicated in various biological processes, including cellular differentiation, stem-cell self-renewal, cell tissue development, circadian-rhythm regulation, response to ultraviolet-induced DNA damage, T-cell homeostasis maintenance, the postnatal development of the mouse cerebellum, the modulation of the innate immune response, the promotion of mouse fertility, and the facilitation of dendritic cell antigen presentation (29). The crucial regulatory functions of RBPs in cell survival, migration, and death have been well-documented (30). This study presents the first documented evidence of a new m^{6}A mutation of NOVA2 in HCC. This study showed that the expression of NOVA2 was modulated by a m^{6}A modification in the mRNA. Further, our findings indicated that the primary enzyme responsible for regulating NOVA2 mRNA levels through m^{6}A modification is METTL3. The m^{6}A alteration affects several aspects of mRNA, including nuclear export, splicing, stability, and the translation of certain target mRNAs. These effects are contingent on the specific proteins involved in the recognition process, such as YTHDF1, YTHDF2, and YTHDF3 (18). This study also showed that the METTL3-induced overexpression of NOVA2 mRNA slowed down the degradation of NOVA2 mRNA, which is dependent on the m^{6}A reader protein YTHDF1. This study was also the first to establish the association between m^{6}A alteration and the deregulation of NOVA2 in HCC. There are various types of RNA methylation modifications, and in future research, we will delve deeper into whether NOVA2 are affected by other types of RNA methylation modifications.

Lenvatinib, an orally administered multi-kinase inhibitor, has been approved as a first-line therapy for HCC (31,32). However, a significant number of patients with advanced HCC develop resistance to lenvatinib progression shortly after the treatment is initiated. Thus, the underlying biological mechanisms that contribute to the development of resistance to lenvatinib need to be investigated, and reliable biomarkers that may accurately predict patients' response to lenvatinib treatment need to be identified.

This study showed that HCC cells with increased expression levels of NOVA2 exhibited reduced sensitivity to lenvatinib. In addition, 30 HCC patients who had surgery and received adjuvant lenvatinib exhibited elevated levels of NOVA2 expression had reduced overall survival after receiving lenvatinib treatment. Thus, the expression of NOVA2 in HCC tumors should be considered to identify individuals who may derive therapeutic benefits from lenvatinib treatment.

Conclusions

NOVA2 is involved in the process of liver CSC self-renewal and carcinogenesis. In addition, NOVA2 is associated with lenvatinib resistance in HCC patients. Thus, NOVA2 expression may help identify patients with a higher chance of benefiting from lenvatinib treatment and can be a promising therapeutic target for HCC. This study owned its limitations, as two different types of samples are used-patients and cell lines models. The use of cell lines does not allow to understand how the tumoural microenvironment may interfere with treatment response in this study.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Eastern Hepatobiliary Surgery Hospital, Naval Medical University (No. EHBHKY2024-K014-P001), and informed consent was taken from all the patients.

References

1. Ganesan P, Kulik LM. Hepatocellular Carcinoma: New Developments. Clin Liver Dis 2023;27:85-102.
2. Liu X, Yang W, Petrick JL, et al. Higher intake of whole grains and dietary fiber are associated with lower risk of liver cancer and chronic liver disease mortality. Nat Commun 2021;12:6388.
3. Xiang DM, Sun W, Zhou T, et al. Oncofetal HLF transactivates c-Jun to promote hepatocellular carcinoma development and sorafenib resistance. Gut 2019;68:1858-71.
4. Cappuyns S, Corbett V, Yarchoan M, et al. Critical Appraisal of Guideline Recommendations on Systemic Therapies for Advanced Hepatocellular Carcinoma: A Review. JAMA Oncol 2024;10:395-404.
5. You Q, Li R, Yao J, et al. Insights into lenvatinib resistance: mechanisms, potential biomarkers, and strategies to enhance sensitivity. Med Oncol 2024;41:75.
6. Rimini M, Kudo M, Tada T, et al. Nonalcoholic steatohepatitis in hepatocarcinoma: new insights about its prognostic role in patients treated with lenvatinib. ESMO Open 2021;6:100330.
7. Yılmaz M, Kaplan F, Mender I, et al. Cancer Stem Cells and Anti-tumor Immunity. Curr Stem Cell Res Ther 2023;18:445-59.
8. Dianat-Moghadam H, Sharifi M, Salehi R, et al. Engaging stemness improves cancer immunotherapy. Cancer Lett 2023;554:216007.
9. Liu Y, Zhang C, Fan J, et al. Comprehensive analysis of clinical significance of stem-cell related factors in renal cell cancer. World J Surg Oncol 2011;9:121.
10. Gao Y, You M, Fu J, et al. Intratumoral stem-like CCR4+ regulatory T cells orchestrate the immunosuppressive microenvironment in HCC associated with hepatitis B. J Hepatol 2022;76:148-59.
11. Salvadori G, Zanardi F, Iannelli F, et al. Fasting-mimicking diet blocks triple-negative breast cancer and cancer stem cell escape. Cell Metab 2021;33:2247-2259.e6.
12. Lai SA, Gundlapalli H, Ekiz HA, et al. Blocking Short-Form Ron Eliminates Breast Cancer Metastases through Accumulation of Stem-Like CD4+ T Cells That Subvert Immunosuppression. Cancer Discov 2021;11:3178-97.
13. Bayik D, Lathia JD. Cancer stem cell-immune cell crosstalk in tumour progression. Nat Rev Cancer 2021;21:526-36.
14. Xiang D, Cheng Z, Liu H, et al. Shp2 promotes liver cancer stem cell expansion by augmenting β-catenin signaling and predicts chemotherapeutic response of patients. Hepatology 2017;65:1566-80.
15. Sommer G, Heise T. Role of the RNA-binding protein La in cancer pathobiology. RNA Biol 2021;18:218-36.
16. Knupp D, Cooper DA, Saito Y, et al. NOVA2 regulates neural circRNA biogenesis. Nucleic Acids Res 2021;49:6849-62.
17. Meldolesi J. Alternative Splicing by NOVA Factors: From Gene Expression to Cell Physiology and Pathology. Int J Mol Sci 2020;21:3941.
18. Wang J, Yu H, Dong W, et al. N6-Methyladenosine-Mediated Up-Regulation of FZD10 Regulates Liver Cancer Stem Cells' Properties and Lenvatinib Resistance Through WNT/β-Catenin and Hippo Signaling Pathways. Gastroenterology 2023;164:990-1005.
19. Najafi M, Mortezaee K, Majidpoor J. Cancer stem cell (CSC) resistance drivers. Life Sci 2019;234:116781.
20. Katoh M, Katoh M. WNT signaling and cancer stemness. Essays Biochem 2022;66:319-31.
21. Liu ZX, Li LM, Sun HL, et al. Link Between m6A Modification and Cancers. Front Bioeng Biotechnol 2018;6:89.
22. Corley M, Burns MC, Yeo GW. How RNA-Binding

Proteins Interact with RNA: Molecules and Mechanisms. Mol Cell 2020;78:9-29.
23. Gerstberger S, Hafner M, Tuschl T. A census of human RNA-binding proteins. Nat Rev Genet 2014;15:829-45.
24. Re A, Joshi T, Kulberkyte E, et al. RNA-protein interactions: an overview. Methods Mol Biol 2014;1097:491-521.
25. Qin H, Ni H, Liu Y, et al. RNA-binding proteins in tumor progression. J Hematol Oncol 2020;13:90.
26. Sendinc E, Shi Y. RNA m6A methylation across the transcriptome. Mol Cell 2023;83:428-41.
27. Deng LJ, Deng WQ, Fan SR, et al. m6A modification: recent advances, anticancer targeted drug discovery and beyond. Mol Cancer 2022;21:52.
28. He L, Li H, Wu A, et al. Functions of N6-methyladenosine and its role in cancer. Mol Cancer 2019;18:176.
29. Fang Z, Mei W, Qu C, et al. Role of m6A writers, erasers and readers in cancer. Exp Hematol Oncol 2022;11:45.
30. Popper B, Scheidt T, Schieweck R. RNA-binding protein dysfunction in neurodegeneration. Essays Biochem 2021;65:975-86.
31. Gordan JD, Kennedy EB, Abou-Alfa GK, et al. Systemic Therapy for Advanced Hepatocellular Carcinoma: ASCO Guideline. J Clin Oncol 2020;38:4317-45.
32. Han T, Zheng H, Zhang J, et al. Downregulation of MUC15 by miR-183-5p.1 promotes liver tumor-initiating cells properties and tumorigenesis via regulating c-MET/PI3K/AKT/SOX2 axis. Cell Death Dis 2022;13:200.

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