

RESEARCH

Open Access



Targeting NETO2 suppresses cell proliferation, invasion, and migration and inactivates the STAT3/C-MYC pathway in hepatocellular carcinoma

Na Shun Meng He^{1†}, Xinghua Wu^{2†}, Shu Chen¹, Xinyi Yun¹, Shun Yao¹ and Hai Yu^{1*}

Abstract

Background Neuropilin and tolloid-like 2 (NETO2) facilitates the progression of various cancers, but its role in hepatocellular carcinoma (HCC) is not known. This study aimed to assess the potential of targeting NETO2 in HCC and its relationship with the STAT3/C-MYC pathway.

Methods HCC cells (Huh7 and MHCC-97 H) were cultured and transfected with control siRNA (siCtrl), NETO2 siRNA (siNETO2), control overexpression (oeCtrl), or NETO2 overexpression (oeNETO2), with non-transfected cells used as blank controls.

Results NETO2 mRNA and protein expressions were reduced in both Huh7 and MHCC-97 H cells. EdU and CCK-8 assays indicated that cell proliferation was decreased after siNETO2 transfection in Huh7 and MHCC-97 H cells. TUNEL assay found revealed that the cell apoptosis rate was greater after siNETO2 transfection in MHCC-97 H cells, and tended to be greater in Huh7 cells (but the difference was not statistically significant). Transwell invasion assay revealed that the number of invasive Huh7 and MHCC-97 H cells decreased after siNETO2 transfection. Cell scratch assay revealed that the cell migration rate was reduced after siNETO2 transfection in Huh7 cells but was not significantly different in MHCC-97 H cells. Western blotting revealed that p-STAT3 and C-MYC expressions were decreased after siNETO2 transfection in Huh7 and MHCC-97 H cells. Overexpression experiments revealed that cell proliferation and invasion were promoted but that the cell apoptosis rate was reduced after oeNETO2 transfection in Huh7 and MHCC-97 H cells.

Conclusion NETO2 knockdown suppresses HCC cell proliferation, invasion, and migration and inactivates the STAT3/C-MYC pathway, suggesting that NETO2 is a potential target for HCC treatment.

Keywords NETO2, Hepatocellular carcinoma, Proliferation and apoptosis, Invasion and migration, STAT3/C-MYC pathway

[†]Na Shun Meng He and Xinghua Wu contributed equally to this work.

*Correspondence:
Hai Yu
yuhai110920@163.com

¹Minimally Invasive Intervention Department, Peking University Cancer Hospital Inner Mongolia Hospital, No. 42 Zhaowuda Road, Saihan District, Hohhot 100020, China

²Electromyography Center, The Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010030, China



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

As one of the most fatal malignancies, liver cancer constitutes 758 thousand deaths per year globally and 317 thousand deaths per year in China [1, 2], among which hepatocellular carcinoma (HCC) accounts for the majority of cases [3]. The high mortality of HCC is due mainly to the fact that most cases are diagnosed at a late stage, and effective treatment options are limited [4, 5]. Therefore, the identification of novel and effective targets for HCC treatment remains a research hotspot, which could hopefully improve HCC outcomes [6–8].

Neuroilin and tolloid-like 2 (NETO2) was initially recognized as an auxiliary-kainate receptor subunit that modified both the biophysical properties and synaptic localization of the receptors [9, 10]. Recently, NETO2 was also reported to be an oncogene in several cancers [11–15]. For example, NETO2 induces growth and metastasis via PI3K/AKT and ERK signaling in esophageal cancer [11], facilitates cell proliferation and invasion by activating STAT3 signaling in pancreatic cancer [12], and enhances cell invasion and metastasis by promoting PI3K/Akt/NF- κ B/Snail signaling in gastric cancer [13]. In addition, NETO2 has been reported to be dysregulated and a prognostic marker in some cancers, such as gastric cancer, colorectal cancer, and prostate cancer [13–15]. In HCC, NETO2 serves as one of five gene transcriptomic hepatic signatures that can accurately recognize the rapid progression of HCC and independently predict the mortality of HCC [16].

This study aimed to investigate the potential of NETO2 as a treatment target in HCC cells and its relationship with the STAT3/C-MYC pathway.

Methods

Cell culture

HCC cells (Huh7 and MHCC-97 H) were procured from iCell Bioscience (Shanghai, China). These cells were cultured in Dulbecco's modified Eagle's medium (Servicebio, China) supplemented with 10% fetal bovine serum (Servicebio, China) and penicillin–streptomycin solution (Servicebio, China). The cells were cultivated in a carbon dioxide cell incubator (Bluepard, China). The medium was replaced every 72 h (h), and the cells were passaged with trypsin (Servicebio, China).

Transfection

The control siRNA (siCtrl) and NETO2 siRNA (siNETO2) were procured from GenePharma (Shanghai, China). The siRNA sequences used were as follows (5'–3'): siCtrl (sense, UUCUCCGAACGUGUCA CGUTT; antisense, ACGUGACACGUUCGGAGAAT T), siNETO2-1 (sense, GGAGAUUCAUGUGGAUUA ATT; antisense, UUAUCCACAUGAAUCUCCTT), siNETO2-2 (sense, GACCUUUGAUGAACAUUAUT

T; antisense, AUAUUGUUCAUCAAGGUCTT) and siNETO2-3 (sense, GCAACAUGUGCAUCAUAUAT T; antisense, UUAUUGAUGCACAUGUUGCTT). For transfection, cells were plated in a 6-well plate (3×10^5 cells/well) and divided into the blank, siCtrl, siNETO2-1, siNETO2-2, and siNETO2-3 groups. The blank group was cultivated as normal without transfection. The siCtrl, siNETO2-1, siNETO2-2, and siNETO2-3 groups were transfected with siCtrl, siNETO2, siNETO2-2, and siNETO2-3, respectively. Briefly, the siRNA (60 pmol) was added to 200 μ L of serum-free medium, and then 10 μ L of siRNA-mate (GenePharma, China) was added. The mixture was allowed to stand for 10 min at room temperature (RT) prior to being introduced into the cultured cells. At 48 h after transfection, Huh7 and MHCC-97 H cells were subsequently harvested for quantitative polymerase chain reaction (qPCR) to assess the efficiency of siRNA interference, and siNETO2-2 (re-named siNETO2 in the following experiments) was chosen for further experimentation.

In addition, the NETO2 overexpression (oeNETO2) and negative control overexpression (oeCtrl) plasmids were constructed by GenePharma (Shanghai, China). For transfection, cells were plated in a 6-well plate (3×10^5 cells/well) and divided into blank, oeCtrl, and oeNETO2 groups. The blank group was cultivated as normal without transfection. The oeCtrl and oeNETO2 groups were transfected with the oeCtrl or oeNETO2 plasmid. In brief, cells were cultured and transfected with 4 μ g of plasmid using Lipofectamine 2000 (Thermo Fisher, USA). The cell medium was replaced after 6 h of treatment, and the cells were harvested for further experimentation after being cultured for an additional 48 h.

qPCR

After transfection, Huh7 and MHCC-97 H cells (1×10^6 cells) were incubated with 1 mL of TRIzol (Servicebio, China) for 5 min at RT. Then, 100 μ L of RNA Extraction Buffer (Beyotime, China) was added, and the mixture was vortexed for 15 sections (s), followed by standing for 5 min. After centrifugation ($12000 \times g$, 15 min), the supernatant fluid was carefully isolated and subsequently mixed with isopropanol (Hushi, China). After standing for 10 min, total RNA was collected after centrifugation and washing. After that, 1 ng of total RNA was added to reverse transcribed components (Servicebio, China) and incubated for 10 min at 65 °C. The cDNA template and the components of the qPCR mixture (Servicebio, China) were mixed, and a further qPCR program was carried out. The primers used were as follows (5'–3'): NETO2 (forward: TGCTCGGTCCTCAAAGTGTT, reverse: CC AAATGCCACACTGGGTG) and GAPDH (forward: G AAAGCCTGCCGGTGACTAA, reverse: GCCCAATAC GACCAAATCAGAGA).

Western blotting

After transfection, Huh7 and MHCC-97 H cells (5×10^6 cells) were lysed with 250 μ L of RIPA buffer (Servicebio, China) supplemented with protease inhibitor (Servicebio, China). After 20 min of incubation, the lysate was centrifuged (10000 g, 10 min), which enabled the collection of the protein-containing supernatant. The protein concentration was assessed via a Bicinchonic Acid Kit (Servicebio, China). The proteins were subsequently separated via electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked and incubated with primary antibodies against NETO2, p-STAT3, STAT3, C-MYC (Proteintech, China), and GAPDH (Servicebio, China). These membranes were subsequently incubated with secondary antibodies (Servicebio, China). Finally, the blots were visualized via Super ECL Reagent (Servicebio, China).

5-Ethynyl-2'-deoxyuridine (EdU) staining

After transfection, Huh7 and MHCC-97 H cells were plated into 24-well plates (6×10^4 cells/well) for 24 h. An EdU working solution (Servicebio, China) was prepared and added to the cells (10 μ M) for 2 h. Subsequently, the cells were washed and blocked, followed by incubation with the Click additive solution (Servicebio, China) for 30 min at RT. Nuclei were visualized through 4',6-diamidino-2-phenylindole (DAPI) (Beyotime, China) staining. Finally, photos were taken via a fluorescence microscope (Motic, China).

Cell counting Kit-8 (CCK-8) assay

Following transfection, Huh7 and MHCC-97 H cells were plated in 96-well plates (3×10^3 cells/well). After being cultivated for 24 h, 10 μ L of CCK-8 solution (Servicebio, China) was added to each well for 2 h. Subsequently, the optical density (OD) value was read via a microplate reader (Huadong Electronics, China).

Terminal-deoxynucleotidyl transferase-mediated Nick-End labeling (TUNEL)

After transfection, Huh7 and MHCC-97 H cells were dispensed into 24-well plates (6×10^4 cells/well). After a 24-h period of cultivation, the cells were washed and fixed with paraformaldehyde (Servicebio, China) and then stained with TUNEL staining solution (Servicebio, China) for 1 h at RT. Subsequently, DAPI staining was performed, and photos were taken.

Transwell invasion assay

Transwell assays were conducted to measure the invasive potential of Huh7 and MHCC-97 H cells post transfection. First, the cells were cultured without serum for 12 h and then plated in a Transwell insert (Corning, USA). Standard medium was added to the lower chamber.

Following 24 h of incubation, invasive cells that migrated through the membrane were visualized via crystal violet (Servicebio, China) staining.

Cell scratch assay

Cell migration after transfection was assessed with a cell scratch assay. In brief, Huh7 and MHCC-97 H cells were seeded into 6-well plates (3×10^5 cells/well) and allowed to proliferate until they reached over 90% confluence. The scratches were produced via a pipette tip and photographed (designated the 0 h timepoint). After being cultured for 24 h without serum, the scratches were photographed at the same location (designated the 24 h timepoint).

Analysis of data from **The Cancer Genome Atlas (TCGA)**.

An in-depth analysis of HCC data sourced from the TCGA via the University of Alabama at the Birmingham Cancer Data Analysis Portal (UALCAN) (<https://ualcan.path.uab.edu/>) and the Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/>) online tools, including NETO2 expression in HCC compared with normal controls, NETO2 expression across various stages of tumors, and the potential association of NETO2 expression with overall survival, was performed.

Statistical analysis

One-way analysis of variance (ANOVA) with Tukey's test was used to compare the differences among groups. Statistical analyses were conducted via GraphPad 9.0 software (GraphPad, USA). A *P* value < 0.05 was considered significant.

Results

SiNETO2 transfection

Three siRNAs were designed and tested for the knock-down efficiency, which revealed that 3 siRNAs all strongly repressed the NETO2 expression, among which the effect of siNETO2-2 was the best, therefore chosen for the latter experiments of siRNA interference (renamed siNETO2 in the following experiments) (Supplementary Fig. 1).

After the cells were transfected with siNETO2, NETO2 mRNA expression was greatly reduced in both Huh7 and MHCC-97 H cells (Fig. 1A). Moreover, NETO2 protein expression was also obviously decreased after siNETO2 transfection in Huh7 and MHCC-97 H cells (Fig. 1B-C).

SiNETO2 reduced cell proliferation

Two methods were used to detect cell proliferation after siNETO2 transfection: EdU and CCK-8. EdU assays revealed that the number of positively stained Huh7 and MHCC-97 H cells was lower after siNETO2 transfection (Fig. 2A-B). The results of the CCK-8 assay suggested that

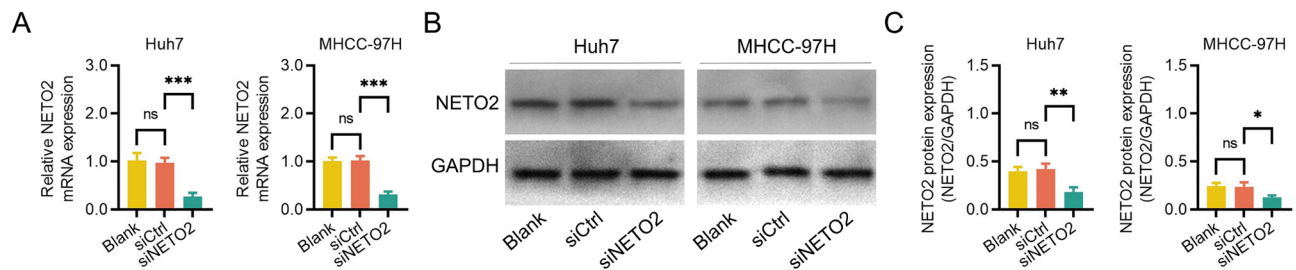


Fig. 1 Efficiency of siNETO2 transfection in HCC cells. Comparison of NETO2 mRNA expression (A), NETO2 western blotting image (B), and NETO2 protein expression (C) in Blank group, siCtrl group, and siNETO2 group

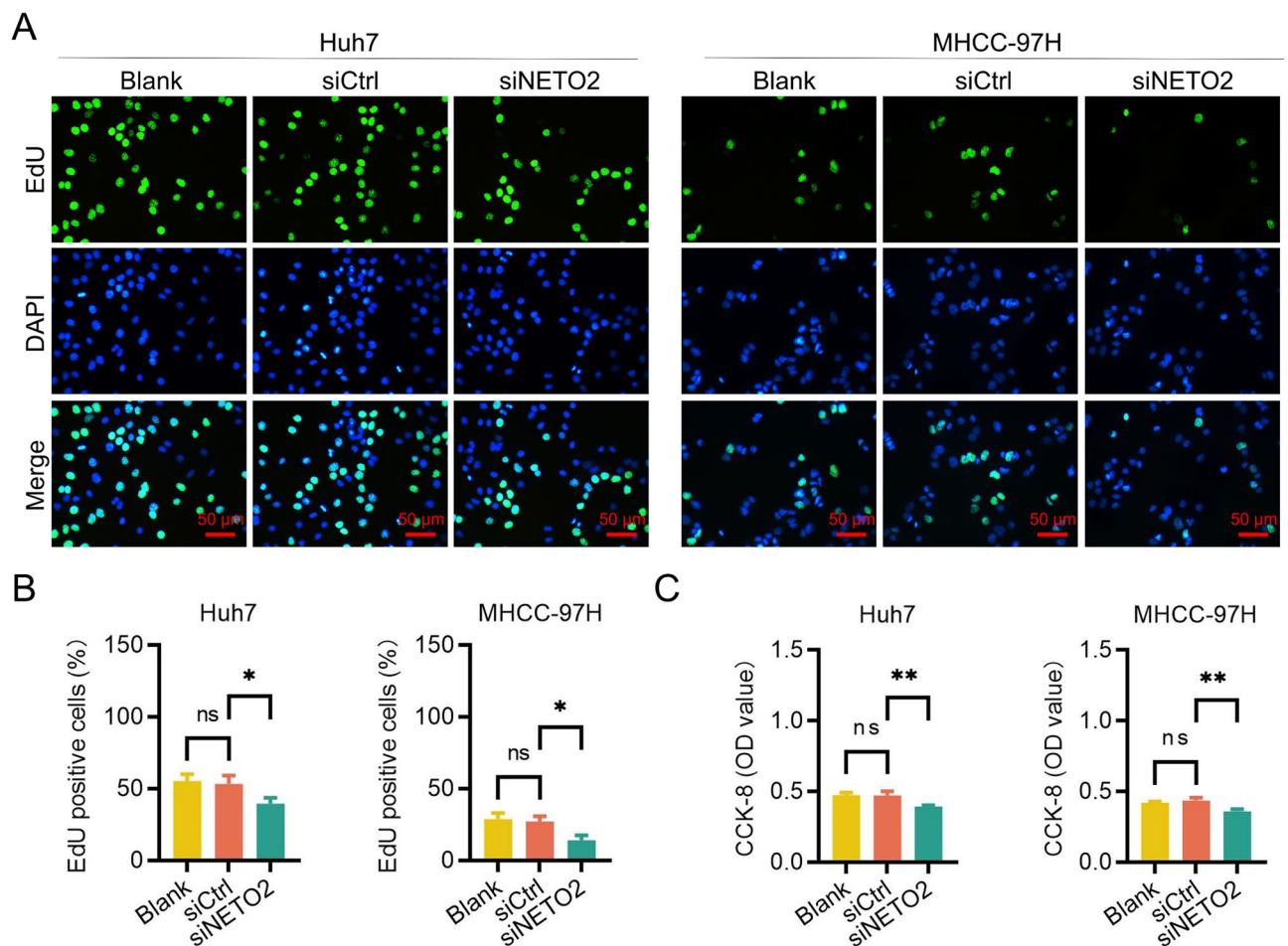


Fig. 2 Effect of siNETO2 on cell proliferation in HCC cells. Comparison of EdU staining image (A), EdU positive staining cells (B), and OD value by CCK-8 (C) in Blank group, siCtrl group, and siNETO2 group

the OD value was also lower after siNETO2 transfection in Huh7 and MHCC-97 H cells (Fig. 2C).

SiNETO2 promoted cell apoptosis to some degree

A TUNEL assay was performed to detect cell apoptosis, which revealed that the cell apoptosis rate tended to increase after siNETO2 transfection in Huh7 cells, but was not significant. However, the cell apoptosis rate

was significantly greater after siNETO2 transfection in MHCC-97 H cells (Fig. 3A-B).

SiNETO2 decreased cell invasion and migration

Transwell invasion assay was performed to detect cell invasion, which revealed that the number of invasive Huh7 and MHCC-97 H cells decreased after siNETO2 transfection (Fig. 4A-B). A cell scratch assay was subsequently performed to detect cell migration, which

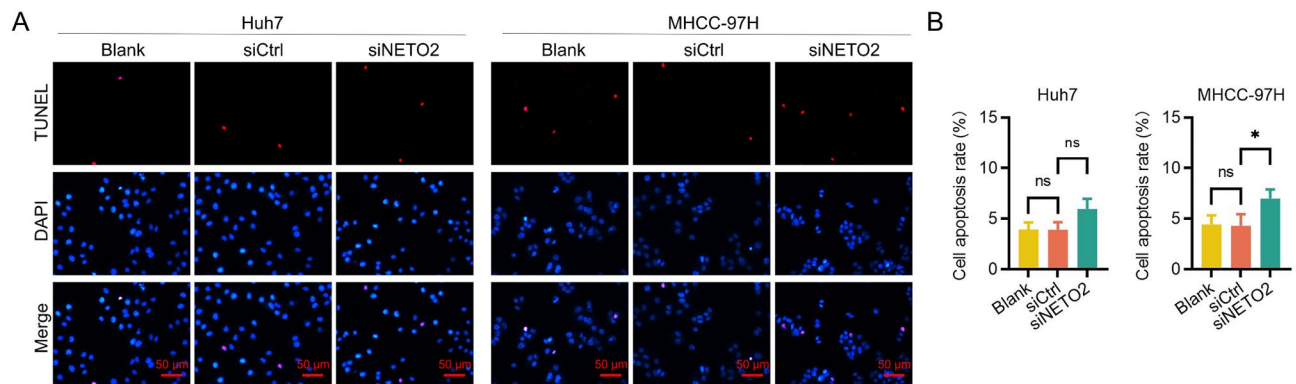


Fig. 3 Effect of siNETO2 on cell apoptosis in HCC cells. Comparison of TUNEL staining image (A), and cell apoptosis rate (B) in Blank group, siCtrl group, and siNETO2 group

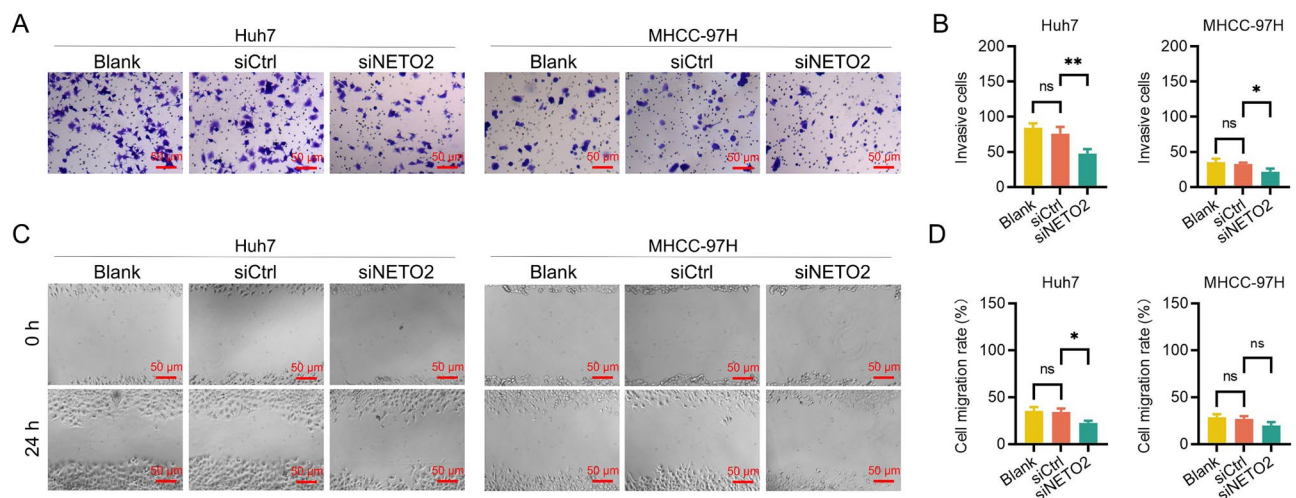


Fig. 4 Effect of siNETO2 on cell invasion and migration in HCC cells. Comparison of Transwell invasion image (A), invasive cells (B), cell scratch image (C), and cell migration rate (D) in Blank group, siCtrl group, and siNETO2 group

revealed that the cell migration rate was reduced after siNETO2 transfection in Huh7 cells, but was not significantly different in MHCC-97 H cells (Fig. 4C-D).

SiNETO2 inactivated the STAT3/C-MYC pathway

The STAT3 pathway was previously reported to be regulated by NETO2, the latter enhanced cell proliferation and invasion by inactivating the STAT3 pathway in pancreatic cancer [12]; therefore, the STAT3/C-MYC pathway was subsequently detected. Western blotting was used to evaluate the activation of the STAT3/C-MYC pathway (Fig. 5A). p-STAT3 expression was downregulated after siNETO2 transfection in Huh7 cells, but only slightly decreased in MHCC-97 H cells; in addition, C-MYC expression was downregulated after siNETO2 transfection in both Huh7 and MHCC-97 H cells (Fig. 5B).

OeNETO2 promoted cell proliferation and invasion but reduced the cell apoptosis rate

To further explore the effect of NETO2 on HCC cells, oeNETO2 was transfected into the cells. After oeNETO2 transfection, NETO2 expression was strongly increased in both Huh7 and MHCC-97 H cells (Fig. 6A). In addition, the results of the CCK-8 and Transwell assays revealed that cell proliferation and invasion were increased, but the TUNEL assay revealed that the cell apoptosis rate was reduced after oeNETO2 transfection in Huh7 and MHCC-97 H cells (Fig. 6B-F).

Clinical value of NETO2 expression in HCC

According to the UALCAN and GEPIA public databases, NETO2 expression was increased in HCC compared with normal controls (Fig. 7A), and it was correlated with increased tumor stages in HCC patients (Fig. 7B). However, even though HCC patients with high NETO2 expression tended to have worse overall survival than

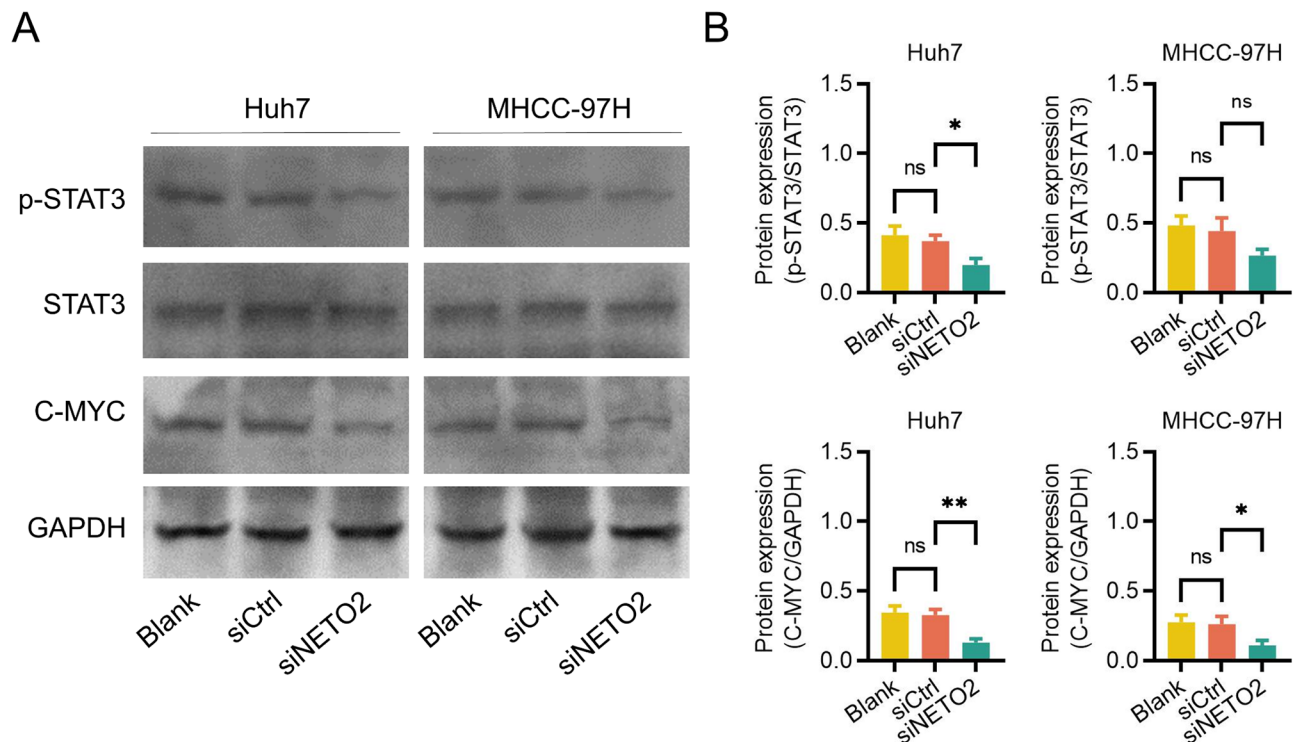


Fig. 5 Effect of siNETO2 on STAT3/C-MYC pathway in HCC cells. Comparison of p-STAT3, STAT3, C-MYC western blotting image (A), and p-STAT3, STAT3, C-MYC protein expressions (B) in Blank group, siCtrl group, and siNETO2 group

patients with low NETO2 expression, this difference was not statistically significant (Fig. 7C).

Discussion

NETO2 is currently known as a factor that regulates the nervous system and tumor biology [9–16]. The former is reflected by the effect of NETO2 on the modification of glutamate receptors and neural transmission and plasticity [9, 10]. The latter is reflected by the impact of NETO2 on regulating cancer viability and mobility and predicting the prognosis of cancers [11–16]. On the basis of the above evidence, targeting NETO2 was speculated to be able to suppress HCC progression, and this study was subsequently performed to explore this topic. This study revealed that siNETO2 strongly reduced the proliferation of HCC cells via two methods (EdU and CCK-8 assays) and in two cell lines (Huh7 and MHCC-97 H cells). This phenomenon could be explained as follows: (1) NETO2 activates STAT3/C-MYC (as identified in the experiments of this study), the latter of which is essential for HCC cell proliferation [17, 18]. (2) NETO2 can also activate several other well-known oncogene pathways, such as the PI3K/AKT, ERK, Wnt, and TGF- β signaling pathways reported in previous studies [11, 19], which are important for HCC cell proliferation [20–23].

This study revealed that siNETO2 promoted cell apoptosis to some degree, as reflected by a significantly greater

percentage of TUNEL-stained MHCC-97 H cells, but a tendency without statistical significance in Huh7 cells. We believe that this phenomenon could be explained by: siNETO2 downregulates Bcl-2 while upregulating C-Caspase 3 to increase cell apoptosis [11]. In addition, the weaker effect of siNETO2 on cell apoptosis than on cell proliferation in HCC may result from the following: although cell proliferation and apoptosis often show opposite trends, proliferation is contributed not only by apoptosis but also by other types of cell death.

With respect to cell mobility, this study revealed that siNETO2 decreased the invasion and migration of HCC cells, as reflected by Transwell invasion and cell scratch assays in two cell lines (Huh7 and MHCC-97 H cells). This phenomenon could be explained as follows: (1) NETO2 promotes STAT3/C-MYC, PI3K/AKT, ERK, Wnt, and TGF- β signaling, as mentioned above [17–23], to promote HCC invasion and migration. (2) NETO2 activates epithelial-mesenchymal transition (EMT) [11], to increase HCC invasion and migration.

Interestingly, this study also explored the effect of oeNETO2 on HCC cells and revealed that oeNETO2 promoted cell proliferation and invasion but repressed the cell apoptosis rate in two HCC cell lines. These findings further validated our hypothesis. Moreover, this study revealed that siNETO2 inactivates the STAT3/C-MYC pathway in HCC cells, which further reveals the potential

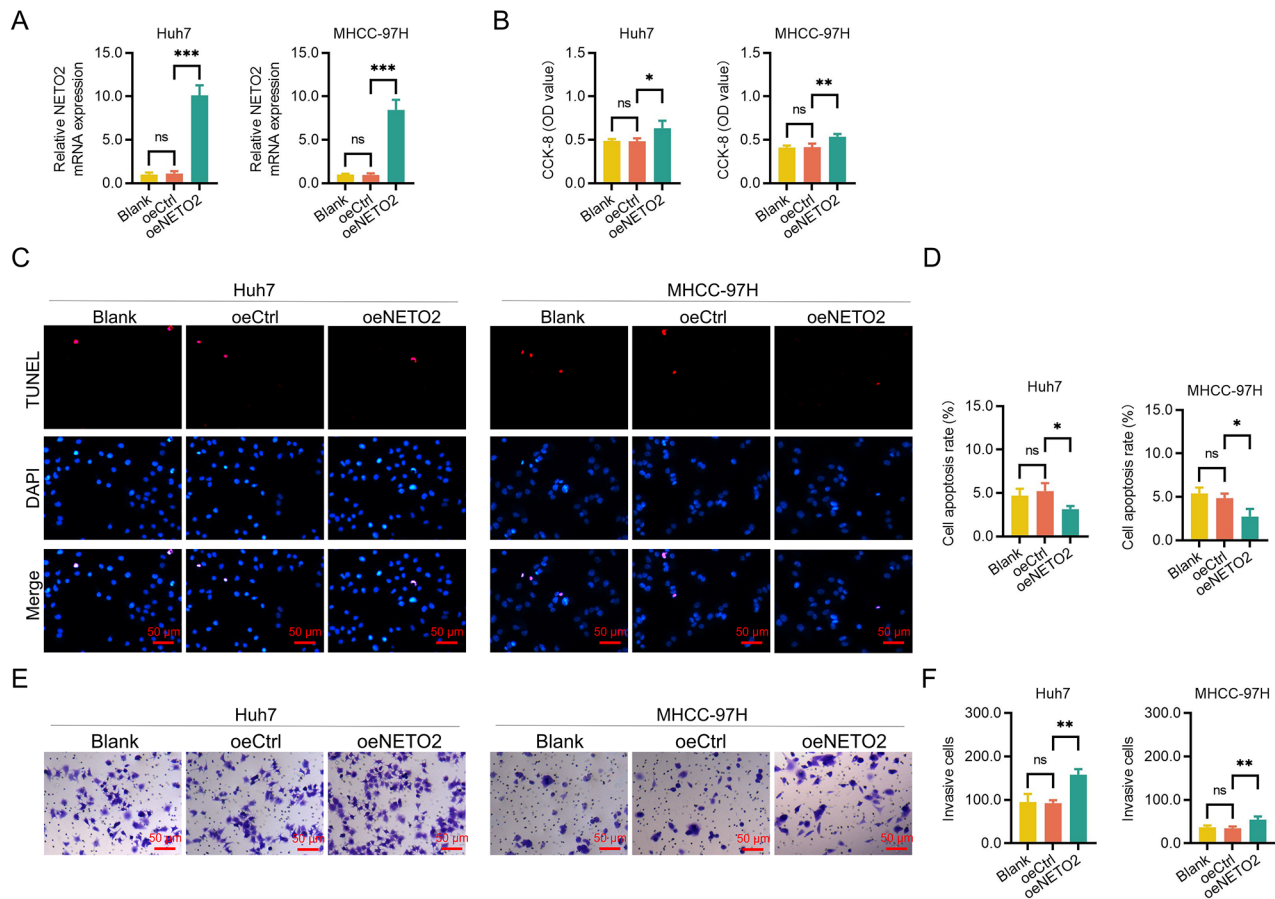


Fig. 6 Effect of oeNETO2 on cell proliferation, apoptosis and invasion in HCC cells. Comparison of NETO2 expression (A), OD value by CCK-8 (B), TUNEL staining image (C), cell apoptosis rate (D), Transwell invasion image (E), and invasive cells (F) in Blank group, oeCtrl group, and oeNETO2 group

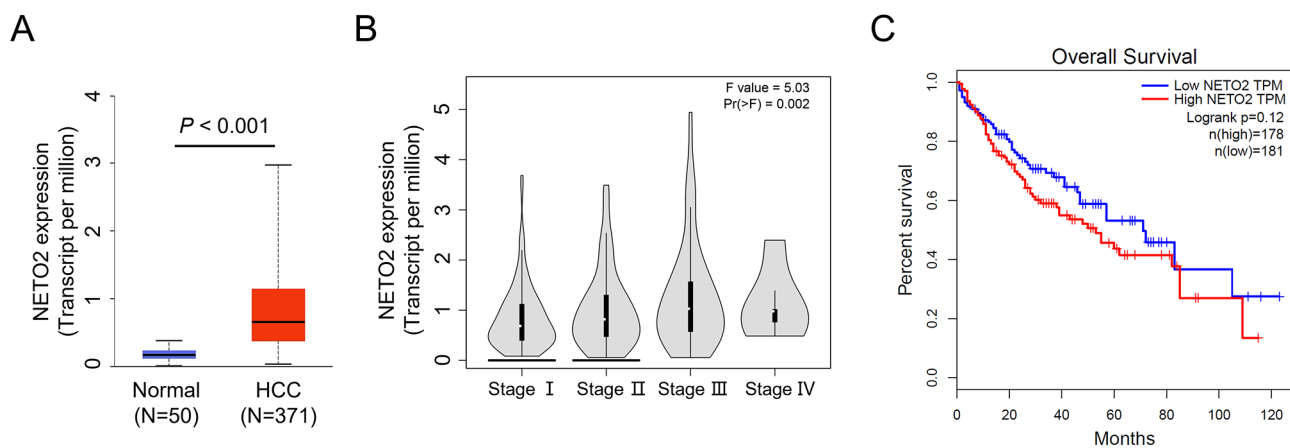


Fig. 7 NETO2 expression in HCC patients. Comparison of NETO2 expression between HCC and normal controls (A). NETO2 expression among different stages of HCC patients (B). Comparison of overall survival between HCC patients with high NETO2 expression and low NETO2 expression (C)

molecular biology of NETO2 in HCC. NETO2 has been reported to promote cell proliferation, invasion and migration by activating STAT3 signaling in pancreatic cancer but has never been reported in HCC [12]. In addition, STAT3/C-MYC signaling is a well-known oncogene pathway that promotes the progression and treatment

resistance of various cancers [18, 24–26]. STAT3/C-MYC signaling has also been reported to promote HCC growth, invasiveness, and oxidative stress [17, 27, 28]. These findings suggest that the effect of NETO2 in HCC may result from its regulation of the STAT3/C-MYC pathway. To further explore the relationship between

NETO2 and the STAT3/C-MYC pathway, this study used the STRING public database (<https://cn.string-db.org/>) to analyze the potential relationship between NETO2 and the STAT3 pathway. As shown in Supplementary Fig. 2, NETO2 could regulate STAT3 via IL9R, IL20, IL27RA, and EBI3. However, further validation, such as animal experiments, is needed in the future.

Conclusions

In conclusion, NETO2 knockdown restrains cell proliferation, invasion, and migration and inactivates the STAT3/C-MYC pathway in HCC, suggesting that NETO2 is a potential target for HCC treatment.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12957-025-03717-1>.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

Not applicable.

Author contributions

Na Shun Meng He: Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing. Xinghua Wu: Conceptualization, Formal analysis, Methodology, Resources, Writing - original draft, Writing - review & editing. Shu Chen: Data curation, Investigation, Resources, Writing - original draft, Writing - review & editing. Xinyi Yun: Data curation, Investigation, Resources, Writing - original draft, Writing - review & editing. Shun Yao: Data curation, Investigation, Resources, Writing - original draft, Writing - review & editing. Hai Yu: Conceptualization, Formal analysis, Methodology, Supervision, Writing - original draft, Writing - review & editing.

Funding

No funding available for this work.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 8 November 2024 / Accepted: 15 February 2025

Published online: 29 March 2025

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–63.
2. Han B, Zheng R, Zeng H, et al. Cancer incidence and mortality in China, 2022. *J Natl Cancer Cent*. 2024;4(1):47–53.
3. Vogel A, Meyer T, Sapichochin G, Salem R, Saborowski A. Hepatocellular carcinoma. *Lancet*. 2022;400(10360):1345–62.
4. Anwanwan D, Singh SK, Singh S, Saikam V, Singh R. Challenges in liver cancer and possible treatment approaches. *Biochim Biophys Acta Rev Cancer*. 2020;1873(1):188314.
5. Reig M, Forner A, Rimola J, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J Hepatol*. 2022;76(3):681–93.
6. Wang Y, Deng B. Hepatocellular carcinoma: molecular mechanism, targeted therapy, and biomarkers. *Cancer Metastasis Rev*. 2023;42(3):629–52.
7. Ajoolabady A, Tang D, Kroemer G, Ren J. Ferroptosis in hepatocellular carcinoma: mechanisms and targeted therapy. *Br J Cancer*. 2023;128(2):190–205.
8. Pessino G, Scotti C, Maggi M, Immuno-Hub C. Hepatocellular Carcinoma: Old and emerging therapeutic targets. *Cancers (Basel)*. 2024;16(5).
9. Tomita S, Castillo PE. Neto1 and Neto2: auxiliary subunits that determine key properties of native kainate receptors. *J Physiol*. 2012;590(10):2217–23.
10. Copits BA, Swanson GT. Dancing partners at the synapse: auxiliary subunits that shape kainate receptor function. *Nat Rev Neurosci*. 2012;13(10):675–86.
11. Xu JC, Chen TY, Liao LT, et al. NETO2 promotes esophageal cancer progression by inducing proliferation and metastasis via PI3K/AKT and ERK pathway. *Int J Biol Sci*. 2021;17(1):259–70.
12. Li Y, Zhang Y, Liu J. NETO2 promotes pancreatic cancer cell proliferation, invasion and migration via activation of the STAT3 signaling pathway. *Cancer Manag Res*. 2019;11:5147–56.
13. Liu JY, Jiang L, He T, et al. NETO2 promotes invasion and metastasis of gastric cancer cells via activation of PI3K/Akt/NF-kappaB/Snail axis and predicts outcome of the patients. *Cell Death Dis*. 2019;10(3):162.
14. Hu L, Chen HY, Cai J, et al. Upregulation of NETO2 expression correlates with tumor progression and poor prognosis in colorectal carcinoma. *BMC Cancer*. 2015;15:1006.
15. Sun Z, Mao Y, Zhang X, et al. Identification of ARHGGEF38, NETO2, GOLM1, and SAPCD2 Associated with prostate Cancer progression by Bioinformatic Analysis and experimental validation. *Front Cell Dev Biol*. 2021;9:718638.
16. Villa E, Critelli R, Lei B, et al. Neoangiogenesis-related genes are hallmarks of fast-growing hepatocellular carcinomas and worst survival. Results from a prospective study. *Gut*. 2016;65(5):861–9.
17. Zhang L, Yang W, Yang J, Sun F. GPRC5A regulates proliferation and oxidative stress by inhibiting the STAT3/Socs3/c-MYC pathway in hepatocellular carcinoma. *J Clin Biochem Nutr*. 2023;73(1):43–51.
18. Sun L, Yan Y, Lv H, et al. Rapamycin targets STAT3 and impacts c-Myc to suppress tumor growth. *Cell Chem Biol*. 2022;29(3):373–85. e6.
19. Fedorova MS, Snezhkina AV, Lipatova AV, et al. NETO2 is deregulated in breast, prostate, and Colorectal Cancer and participates in Cellular Signaling. *Front Genet*. 2020;11:594933.
20. Tian LY, Smit DJ, Jucker M. The role of PI3K/AKT/mTOR signaling in Hepatocellular Carcinoma Metabolism. *Int J Mol Sci*. 2023;24(3).
21. Moon H, Ro SW. MAPK/ERK Signaling Pathway in Hepatocellular Carcinoma. *Cancers (Basel)*. 2021;13(12).
22. Gajos-Michniewicz A, Czyz M. WNT/beta-catenin signaling in hepatocellular carcinoma: the aberrant activation, pathogenic roles, and therapeutic opportunities. *Genes Dis*. 2024;11(2):727–46.
23. Xin X, Cheng X, Zeng F, Xu Q, Hou L. The role of TGF-beta/SMAD signaling in Hepatocellular Carcinoma: from mechanism to Therapy and Prognosis. *Int J Biol Sci*. 2024;20(4):1436–51.
24. Amaya ML, Inguva A, Pei S, et al. The STAT3-MYC axis promotes survival of leukemia stem cells by regulating SLC1A5 and oxidative phosphorylation. *Blood*. 2022;139(4):584–96.
25. Zhang E, Chen Z, Liu W, et al. NCAPG2 promotes prostate cancer malignancy and stemness via STAT3/c-MYC signaling. *J Transl Med*. 2024;22(1):12.
26. Zhang S, Li J, Xie P, et al. STAT3/c-Myc Axis-mediated metabolism alterations of inflammation-related glycolysis involve with colorectal carcinogenesis. *Rejuvenation Res*. 2019;22(2):138–45.
27. Guo D, Gu Y, Ma D, et al. A novel microRNA miR-MTCO3P38 inhibits malignant progression via STAT3/PTTG1/MYC in hepatocellular carcinoma. *Genes Dis*. 2022;9(4):845–48.
28. Youness RA, El-Tayebi HM, Assal RA, Hosny K, Esmat G, Abdelaziz AI. MicroRNA-486-5p enhances hepatocellular carcinoma tumor suppression

through repression of IGF-1R and its downstream mTOR, STAT3 and c-Myc. *Oncol Lett.* 2016;12(4):2567–73.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.