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Original Research Article

LncRNA NORAD/miR-202-5p regulates hepatoma cell viability, apoptosis via EGFR/PI3K/AKT signaling pathway

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Abstract

Purpose: *To examine the potential modulatory mechanisms of NORAD in hepatoma in vitro.*
Methods: *In this study, four human hepatoma cell lines (Bel7404, PLC5, HepG2, and HuH7), as well as a human immortalized Normal MIHA cell line were employed. Transfection was performed to up- or down-regulate NORAD and miR-202-5p expression in cells. RT-qPCR was used to measure expressions of NORAD, miR-202-5p, and biomarkers for cell cycle and apoptosis in HCC cell lines. CCK-8, Flow Cytometry for apoptosis and cell cycle arrest and Western blot experiments were performed to determine cellular viability, cell cycle arrest and apoptosis and related protein expressions. Bioinformatics tool was used to find possible binding sites on NORAD and miR-202-5p, which were further validated by dual-luciferase reporter gene assays.*
Results: *NORAD adversely modulated miR-202-5p in hepatoma cells and mediated cell viability, apoptosis and cell cycle arrest through regulating miR-202-5p. Functional experiments revealed that downregulation of NORAD or upregulation of miR-202-5p suppressed cell viability and inhibited apoptosis and cell cycle arrest. Silenced NORAD regulated the EGFR/PI3K/AKT pathway via enhancing miR-202-5p expression in HCC cell lines.*
Conclusion: *NORAD interaction with miR-202-5p mediated the EGFR/PI3K/AKT network.*

Keywords*: Apoptosis, Cell viability, EGFR/PI3K/AKT signaling, MiR-202-5p, NORAD*

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a distinct malignancy that results from the onset of chronic liver disease at a speed conditional to the multifarious interactions within the host and environmental factors [1]. Recent findings revealed that alcoholism, tobacco smoking, and obesity are common factors that escalate the risk of HCC, which enable practitioners to discover a high-risk group for HCC among patients with cirrhosis [2]. Other researchers have also identified risk factors for HCC that include chronic liver infections, like hepatitis B or C viruses, nonalcoholic fatty liver disease, and consumption of aflatoxins [3].

Presently, a selection of findings has recommended that non-coding RNAs cause hepatoma carcinogenesis, pointing out the possibility of lncRNAs as valuable biomarkers for cancer prediction, identification and therapy [4,5]. LncRNA GAS6-AS2 was also discovered to expedite tumor development and metastasis of HCC through activation of PI3K/AKT/FoxO3a regulatory network[6]. Several other lncRNAs, such as Hox transcript antisense intergenic RNA (HOTAIR)[7], HAND2-AS1[8], linc00467[9], etc. have been described to act oncogenic or suppressive functions in HCC progression. NORAD, a non-coding RNA which is activated by DNA damage, also known as LINC00657, has been reported to be upregulated and stimulate the metastasis of HCC [10]. However, underlying molecular mechanism of NORAD regarding cell apoptosis and proliferation in hepatoma still remains unclear.

The expression patterns and functional roles of NORAD/miR-202-5p in non-small cell lung cancer (NSCLC) were investigated revealed [11]. MiR-202-5p was investigated as tumor suppressor in colorectal cancer (CRC) with outcomes showing miR-202-5p was lowly-expressed in CRC tissues, and inhibited CRC progression via targeting UHRF1 [12] and by directly targeting SMARCC1 [13]. MiR-202-5p suppressed bladder cancer development through EGFR pathway[14]. However, the fundamental molecular functions of miR-202-5p in hepatoma, coupled with NORAD interaction and EGFR/PI3K/AKT signaling pathway remain unknown. The down-regulating activity of EGFR and PI3K/AKT signaling pathway has been reported via interaction with Ganglioside GM3, which inhibited hepatoma cell motility[15]. Therefore, this study was aimed to investigate the fundamental functions of NORAD/miR-202-5p/EGFR/PI3K/AKT axis in the regulation of hepatoma cell viability, apoptosis, employing an HCC cell model.

METHODS

Cell culture

The hepatocellular carcinoma human cells, HepG2, Bel7404, PLC5, and HuH7, as well as a human immortalized Normal MIHA (MIHA) cell line were obtained from the Cell Systems (Seattle, USA). Cells were cultured in RPMI-1640 with ten percent FBS and placed in a lab cell incubator at 37 °C with 5 % CO_2 (ThermoFisher, USA).

Cell transfection

Transfection was conducted in HepG2 and HuH7 cells using Lipofectamine 3000 as per the product protocols (ThermoFisher, USA). Specifically, pcDNA3.1-NORAD, short hair RNA (sh-NORAD), miR-202-5p mimics and their controls were provided by Gene Pharma (Shanghai, China).

RT-qPCR

Total RNAs were isolated and extracted from the HCC cells using Beyozol Kit and BeyoRT™ cDNA First Chain Kit was used for reverse transcription. RT-qPCR assay was conducted using Beyofast™ SYBR Green QPCR and miRNA qRT-PCR detection kit. All the kits mentioned were bought from Beyotime, Shanghai, China. GAPDH/U6 were used as normalized control and the $2^{-\Delta\Delta CT}$ analysis was for relative expression levels of NORAD and miR-202-5p. All primers were produced by Sangon, China. And the sequences for pirmers were included in Table 1.

Table 1: Primer sequences

Target genes	Forward/ Reverse	Sequences
NORAD	Forward	TGATAGGATACATCTT GGACATGGA
NORAD	Reverse	AACCTAATGAACAAGT CCTGACATACA
GAPDH	Forward	GGAGCGAGATCCCTC CAAAAT
GAPDH	Reverse	GGCTGTTGTCATACTT CTCATGG
miR-202-5p	Forward	ACACTCCAGCTGGGTT TCTTCATATACGT
miR-202-5p	Reverse	TGGTGTCGTGGAGTCG
U6	Forward	GCTTCGGCAGCACATA TACTAAAAT
U6	Reverse	CGCTTCACGAATTTGC GTGTCAT
Cyclin D1	Forward	TGCTTGGGAAGTTGTG TTGG
Cyclin D1	Reverse	AATGCCATCACGGTCC CTAC
CDK4	Forward	CTTCCCATCAGCACAG TTCG
CDK4	Reverse	CCTTGATCTCCCGGTC AGTT
Caspase 3	Forward	TGGGCCTGAAATACCA AGTC
Caspase 3	Reverse	CACCCCCAATCATTCC TCTA

Cell Counting Kit-8 method

The cells transfected with either pcDNA3.1-NORAD, control pcDNA3.1 vectors, sh-NC, sh-NORAD, or miR-202-5p mimics were collected

and placed into 96-well plates with 4000 cells in each well. The viability was detected using the Beyotime cell counting kit. Absorbance values in each group were read on a laboratory microplate reader at 450nm (MK3, ThermoFisher, USA)

Cell cycle and apoptosis examination

Flow cytometry methods (FCM) were used to examine the influence of pcDNA3.1-NORAD, sh-NORAD, miR-202-5p mimics on cell cycle distribution and apoptosis. Cells were collected and then fixated with 70 % ethanol and placed in -20 ^{o}C refrigerator. The experiments were performed as previously described [16]. A lab flow cytometer (BD FACSC anto II, BD Biosciences, USA) and corresponding BD FACSDiva software (BD Bioscience) were applied for analysis.

Western blotting assay

Cells were lysed in RIPA with PMSF for protein extraction. The SDS-PAGE methods were applied for protein separation, by which the protein was transferred to polyvinylidene fluoride membranes. Then the membranes were sealed with 5% skim milk. Then membranes were incubated overnight at 4 ^{o}C with anti-phospho PI3K, anti-phospho EGFR, anti-phospho AKT, anti-PI3K, anti-EGFR and anti-AKT from Bioss, China at the dilution rate of 1:200. Anti-GAPDH(Bioss) was used at 1:3000. Goat Anti-Rabbit IgG Antibody,HRP Conjugated (1:1000, Bioss) was used as the secondary antibody thereafter. The ECL kit was used before the observance of the blotting bands (Beyotime). Image J was used for the expression analysis of the blotting bands.

Binding sites prediction

On StarBase, the binding sites on miR-202-5p and NORAD were predicted (http://starbase.sysu.edu.cn/).

Dual-luciferase reporter gene experiments

The pGL3 Dual-luciferase Reporter Vector (Promega, CA, USA) was used to build Wild Type (WT) and Mutant Type (MT) of NORAD plasmids as per the producer's protocols. These plasmids were transfected into HepG2 and HuH7 cells, with miR-202-5p mimic or mimic NC using Lipofectamine 3000 (ThermoFisher). After 48 hours, dual-luciferase reporter experiments were performed using a dual-luciferase reporter activity kit from Beyotime.

Statistical analysis

GraphPad Prism 8(GraphPad, USA) was used for data analysis and figure generation. Student's t-test and ANOVA were for comparisons of differences between groups. $P<0.05$.

RESULTS

NORAD adversely modulates miR-202-5p expression in HCC cells

There were high expression levels of NORAD in almost all the HCC cell lines (HepG2, Bel7404, PLC5, and HuH7), compared to the normal MIHA cell line, as shown in Figure 1A. The highest expression levels were observed in the HepG2 and HuH7 cell lines. On the other hand, there were significantly ($P<0.05$) low levels of miR-202-5p in all the HCC cell lines, compared to the control normal cell line (Figure 1B). Notably, the lowest levels of miR-202-5p expressions were observed in the HepG2 and HuH7 cell lines. Therefore, these two cell lines were adopted for subsequent experiments. Since up-regulation of NORAD and down-regulation of miR-202-5p expressions were noticed in human HCC cells, it was envisaged that this interaction might be involved in aggressive tumor growth and advancement. To further confirm this adverse modulation of NORAD on miR-202-5p, NORAD was knocked down by means of employing its specific short hair pin NORADs (shNORAD) with the outcomes demonstrating a dramatic decrease in NORAD expression. Consequently, the fall in the expression by shNORAD dramatically promoted miR-202-5p expression in both HepG2 and HuH7 cell lines (Figure 1C). In contrast, miR-202-5p was dramatically knocked down in both HepG2 and HuH7 cells after NORAD overexpression (Figure 1D). These data implied that NORAD adversely regulated miR-202-5p expression in human HCC cells.

Silenced NORAD induced cell cycle arrest and apoptosis

A decrease in the expression of NORAD was observed in the shNORAD groups and an increase was noted in oeNORAD groups, compared to the controls (Figure 2A). Then, cellular viability was determined via CCK-8 assay. ShNORAD restrained cellular viability, while pcDNA NORAD enhanced cell survival in HepG2 cells (Figure 2B). Next, the proliferation influence of miR-202-5p in HepG2 and HuH7 cells was probed, with the outcomes demonstrating that miR-202-5p mimics caused an oppressive influence, compared to the

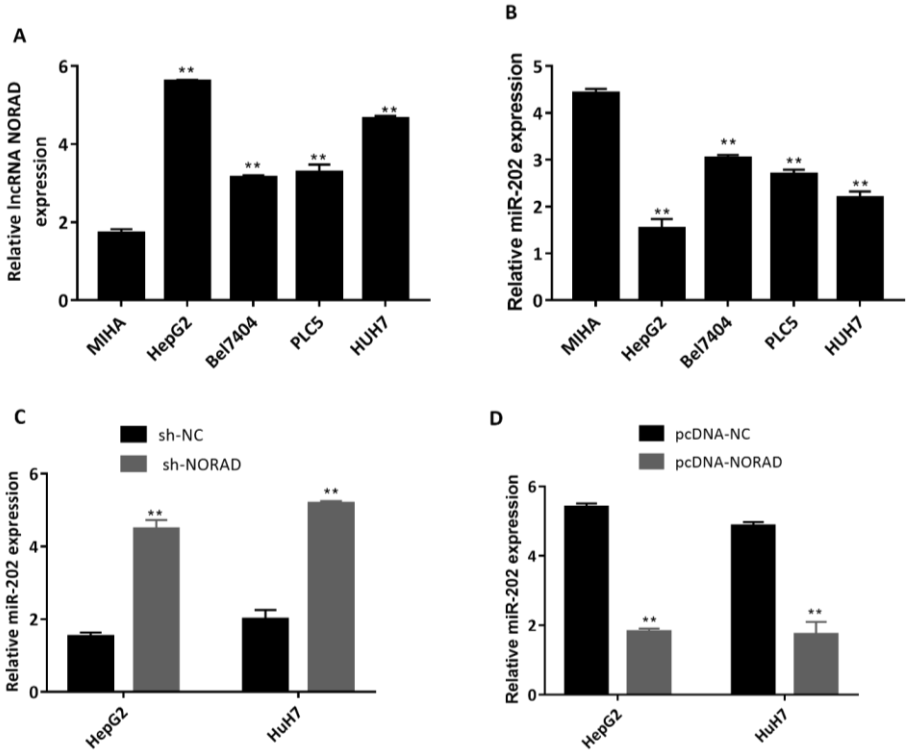

Figure 1: NORAD is upregulated and adversely modulates miR-202-5p expression in hepatoma cells. (A) RT-qPCR evaluated the lncRNA NORAD expression in a group of human HCC cells with the MIHA cell line as a control B) RT-qPCR evaluated the miR-202-5p expression in a group of human HCC cells with the MIHA cell line as a control. RT-qPCR evaluated the modulation of NORAD expression in miR-202-5p:C) miR-202-5p was increased by shNORAD; D) miR-202-5p was knocked down by pcDNA NORAD in HepG2 and HuH7 cells. All expressions were detected by RT-qPCR.

negative control (Figure 2C). Additionally, the cell cycle distribution confirmed that shNORAD stimulated a remarkable increased proportion of HepG2 and HuH7 cells for G1-phase and decreased proportion cells in both S and G2/M phases (Figure 2D), suggesting an existing growth-suppressive effect. The cell cycle distribution also confirmed that miR-202-5p mimics induced significant ($P<0.05$) increased proportion of HepG2 and HuH7 cells for G1-phase and decreased proportion cells in both S and G2/M phases, as presented in Figure 2E, implying that ectopic miR-202-5p promoted growth-suppressive effect. Further cell cycle arrest in G1 phase was validated via RT-qPCR examination of pro-cell cycle markers, cyclin D1 and CDK4 (Figure 2F). As shown in Fig 2F, the expression of pro-cell cycle markers, cyclin D1 and CDK4 was dramatically lowered by shNORAD and also ectopic miR-202-5p, compared to negative controls, confirming an existing growth-suppressive effect in both cells. For the apoptotic rate, NORAD was overexpressed, denoted as pcDNA NORAD and transfected HepG2 and HuH7 cells and performed FCM apoptosis assay. The outcomes revealed a remarkable reduced rate of apoptosis for the pcDNA NORAD, compared with the negative group in both cells (Figure 2G). Further confirmation of this outcome was done via RT-qPCR assay on pro-apoptotic marker, caspase-3 with the results showing significantly ($P<0.05$) decrease in caspase-3 activity in pcDNA NORAD transfected cells, compared to negative control group (Figure2H). In contrast, cell apoptotic rate for miR-202-5p mimics confirmed that miR-202-5p mimics increased apoptosis, compared to the negative control groups for both cell lines (Figure 2G). This was verified by pro-apoptotic marker, caspase-3 which showed a significant ($P<0.05$) increase in cell apoptotic rate in the ectopic miR-202-5p, contrasting the negative controls (Figure 2H). These data implied that silenced NORAD and elevated miR-202-5p expression affected cell cycle arrest and apoptotic rate in HCC cell lines.

NORAD directly targets miR-202-5p in HCC cells

NORAD was predicted to be a target, working together with miR-202-5p via bioinformatics tool Starbase (Figure 3A). Matching to the forecasted sequence, NORAD-3'UTR-WT (wild type) and NORAD-3'UTR-MT (mutant) reporters were generated. The luciferase activity assays

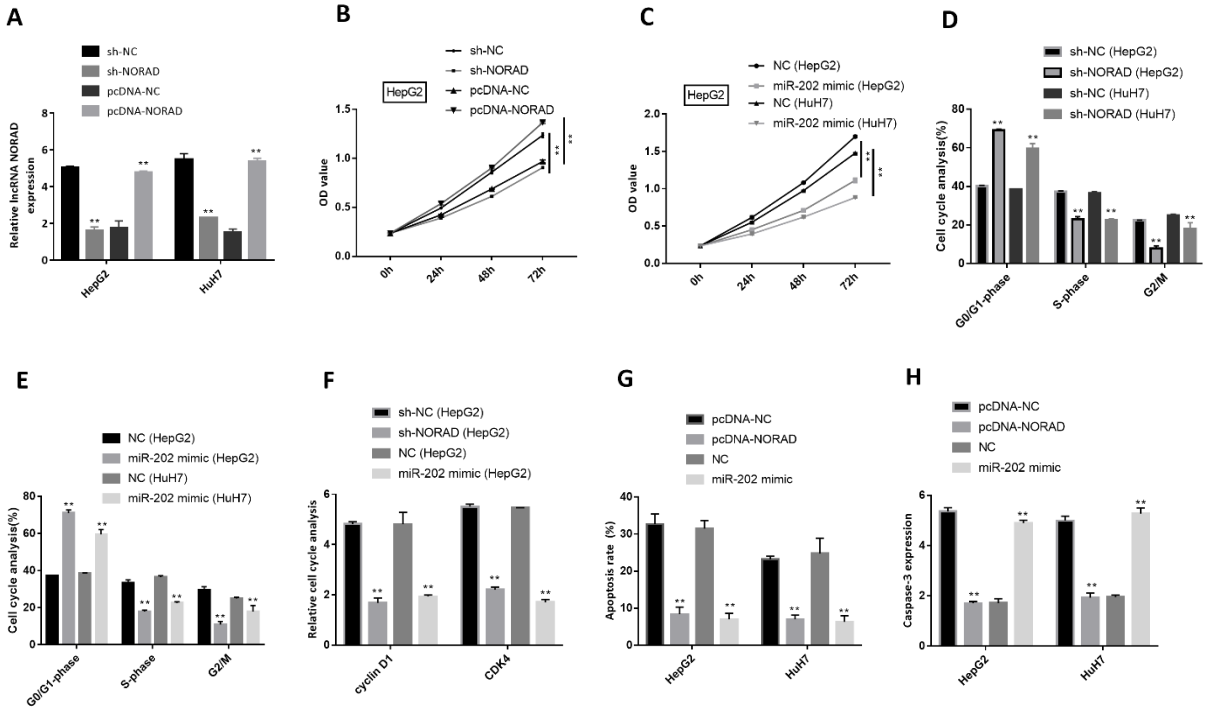

Figure 2: Silenced NORAD induced cell cycle arrest and apoptosis. A) NORAD knockdown or overexpression to determined transfection efficiency in HepG2 and HuH7 cells; B-C) viability in HepG2 and HuH7 cells; D-E) cell cycle analysis; F) analysis of pro-cell cycle markers cyclin D1 and CDK4; G) FCM apoptosis assays; H) Caspase 3 expression

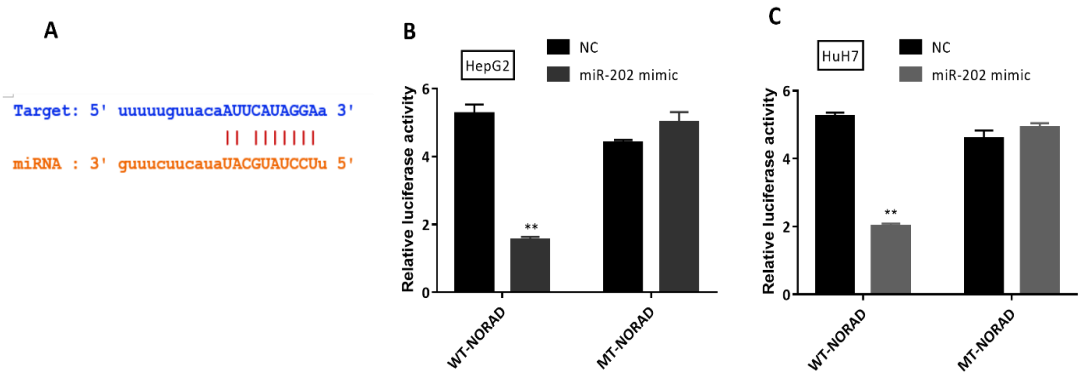

Figure 3: NORAD directly targets miR-202-5p in HCC cells
A) Starbase database was used for target sites prediction; B-C) Luciferase activity analysis in HepG2 and HuH7 cells

demonstrated that endogenous miR-202-5p restrained luciferase activity of the NORAD-3'UTR-WT (wild type) reporter and exerted no change on NORAD-3'UTR-MT (mutant) in both HepG2 and HuH7 cells (Figure 3B and 3C). All together, these data signified that NORAD regulated miR-202-5p to advance HCC carcinogenesis.

MiR-202-5p overturns the cellular viability, cell cycle and inhibitory effects on apoptosis induced by NORAD

To better understand the functional interaction between NORAD and miR-202-5p, RT-qPCR, CCK-8 and FCM assays were performed, respectively. Firstly, the HCC cell lines were transfected with either pcDNA-NC, pcDNA-NORAD+miR-202-5p mimic or pcDNA-NORAD, followed by RT-qPCR to evaluate transfection efficiency. The results obtained as shown in Figure4A suggested an efficient rate of transfection as there was a low expression level of miR-202-5p in NORAD upregulation groups in both HCC cell lines and miR-202-5p expression was restored by miR-202-5p mimic. Then, cell proliferation analysis results (Figure4B and 4C) indicated a decrease in cell viability in the co-

transfected overexpressed-NORAD coupled miR-202-5p mimic group, compared to the NORAD overexpressed group and negative control group in both cell lines. The cell cycle analysis results as shown in Figure 4D and 4E revealed an overturned cell cycle arrest in both HepG2 and HuH7 cells in the G1 phase with increased proportions of cells in S and G2/M phases for the overexpressed-NORAD coupled miR-202-5p mimic group, compared to the NORAD overexpressed group and negative control group. These results were further confirmed by RT-qPCR analysis on pro-cell cycle markers, cyclin D1 and CDK4, in which a decreased expression was observed in the overexpressed-NORAD coupled miR-202-5p mimic group, compared to the NORAD overexpressed and negative control groups in the HepG2 and HuH7 cells (Figure 4F and G). Furthermore， apoptosis outcomes revealed a reversed apoptotic inhibition in the NORAD-overexpressed plus miR-202-5p mimic group, compared to the NORAD overexpressed and negative control groups in the HepG2 and HuH7 cells (Figure 4H). This was also confirmed by caspase-3 expression in the HepG2 and HuH7 cells, as presented in Figure 4I.

NORAD regulates the EGFR/PI3K/AKT signaling via miR-202-5p in HCC cell lines

To further understand the interaction among NORAD/miR-202-5p and EGFR/PI3K/AKT signaling, Western blot assay was performed to detect phosphorylated proteins of the pathways for either sh-NC or shNORAD or shNOARD +miR-202-5p mimic groups in both the HCC cells (HepG2 and HuH7). Western blot examination demonstrated that the HepG2 and HuH7 cells transfected with shNOARD remarkably suppressed p-EGFR, p-PI3K and p-AKT expressions and miR-202-5p mimic synergized with shNORAD to inhibit the p-EGFR, p-PI3K and p-AKT expressions more significantly (Figure 5A). All these data suggested that silenced NORAD interaction with miR-202-5p mimic, by partially regulating the EGFR/PI3K/AKT regulatory network.

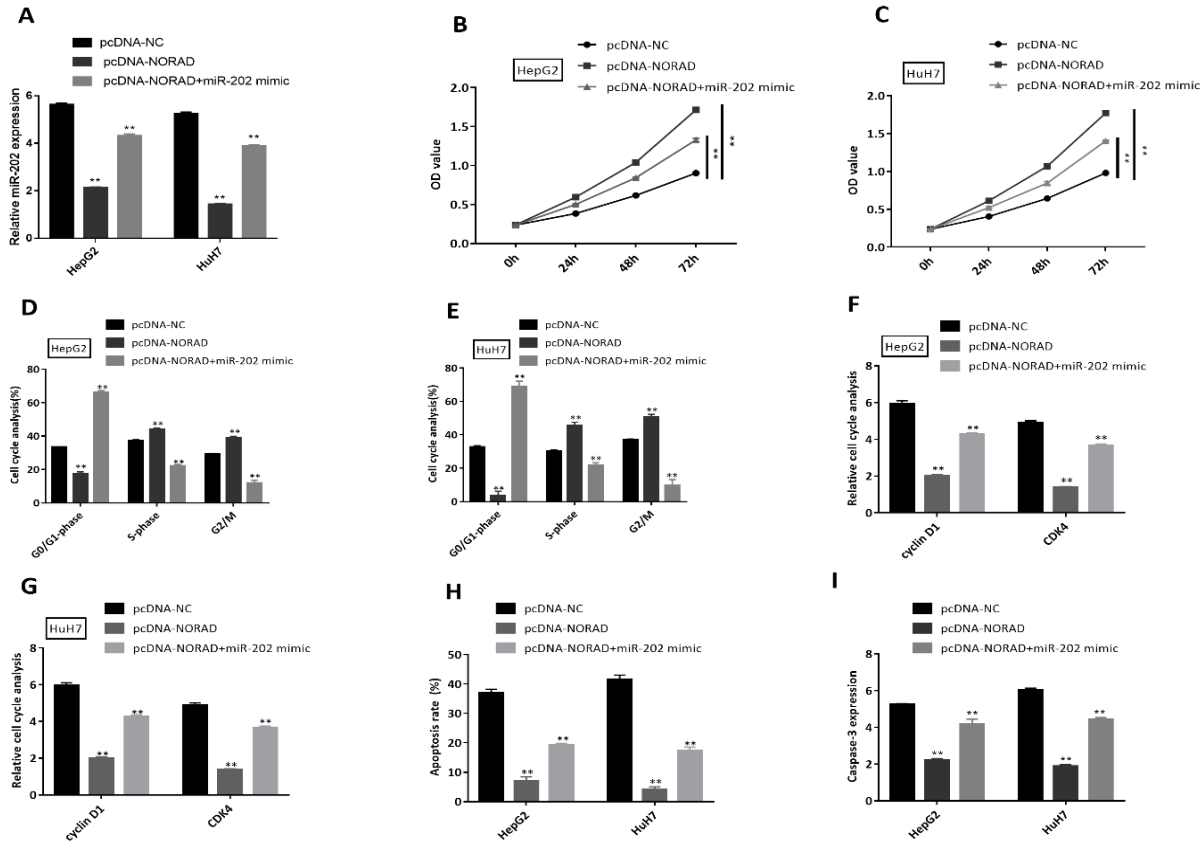

Figure 4: MiR-202-5p overturns cellular viability, cell cycle and inhibitory effects on apoptosis imposed by NORAD**.** A) RT-qPCR determined relative expression of miR-202-5p in either pcDNA-NC, pcDNA-lncRNA NORAD, pcDNA-lncRNA NORAD+miR-202-5p mimic groups; B & C) CCK-8 assay determined cell proliferation analysis; D & E) FCM assay determined cell cycle analysis; F & G) RT-qPCR determined relative expression of pro-cell cycle markers; H) Apoptosis assay determined the apoptosis rate; I) RT-qPCR determined relative expression of pro-apoptosis markers

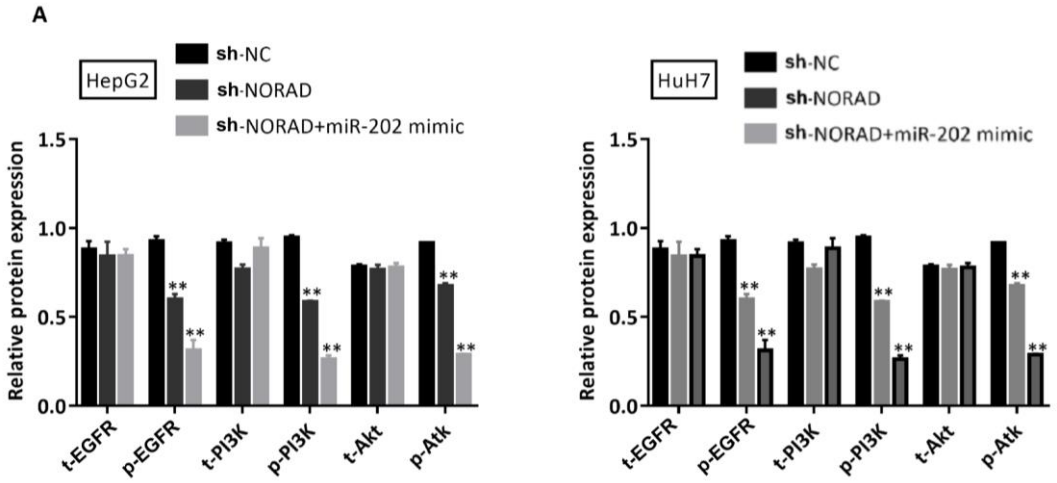

Figure 5: Silenced NORAD regulates the EGFR/PI3K/AKT signaling via enhancing miR-202-5p expression in HCCs. Protein levels of total protein (t-EGFR, t-PI3K, and t-Akt) and phosphorylated proteins of the pathways (p-EGFR, p-PI3K, and p-Akt) were examined via Western blot in: A) HepG2 and B) HuH7 cells.

DISCUSSION

Clarifying the role of translational characteristics in HCC has been taken into account as a solution to disclosing the essential networks in hepatoma oncogenesis. The data in this study have confirmed that NORAD is up-regulated significantly ($P<0.05$) in HCC cells and we have demonstrated that silencing NORAD suppresses cellular growth, initiates cell cycle arrest and enhances apoptosis, eventually prohibiting carcinogenesis.

Recently, some studies have reported that NORAD adversely regulated miR-205 in malignant melanoma and miR-202-5p in papillary thyroid carcinoma[17, 18]. In this study we have established that NORAD adversely regulated miR-202-5p expression in which NORAD overexpression decreasedmiR-202-5p expression; its down-regulation enhanced miR-202-5p expression. The antagonistic influences of NORAD and miR-202-5p on cellular proliferation additionally confirmed this adverse moderation. Moreover, the negative relationship of NORAD and miR-202-5p was also established in hepatoma via direct prediction of binding sites and luciferase activity, which verified this effect. Thus, this negative modulation pattern is coherent with other investigations in esophageal squamous cell carcinoma[19]. As a renowned tumor repressor, in this study, miR-202-5p was found to prevent cellular viability and suppress carcinogenesis in hepatoma *in vitro*. Normally, microRNAs inversely modulate their upstream lncRNA targets in a sequence-particular binding pattern. In this study, the functional experiments have demonstrated that miR-202-5p reversed the cell viability, cell cycle and suppression influence on apoptosis caused by NORAD.

Numerous preceding investigations have reported that activation of PI3K/AKT cell regulatory network is linked to EGFR TKI challenge in non-small cell lung carcinoma[20]. PI3K/AKT is an important downstream network cascade of EGFR, which is overexpressed in NSCLC[21] and hepatoma cell motility[22]. Deregulation of PI3K/AKT regulatory networks was associated with decreased apoptotic rates. In this study, we have shown that interaction between NORAD and miR-202-5p led to reversed advancement in cell viability and improved apoptosis, however, this interaction also had an effect on the signaling pathway. It was observed that the protein phosphorylated levels for the signaling pathway were high in the negative control and silenced NORAD. However, the combined interaction of silenced NORAD and miR-202-5p mimics led to lower phosphorylated levels for the signaling pathway. Thus, the shNORAD+miR-202-5p mimic in HepG2 and HuH7 cells, correspondingly contained decreased p-EGFR, p-PI3K and p-AKT protein expression levels, which implied a partial deactivation of the signaling pathway. This indirectly signifies that inhibiting the EGFR/PI3K/AKT network may perhaps lead to reduced cellular proliferation and improved apoptosis.

CONCLUSION

This study has demonstrated that NORAD acts as an essential gene in hepatoma tumorigenicity by knocking down miR-202-5p to promote carcinogenesis. NORAD promotes HCC cell viability and prevents apoptosis by sponging

miR-202-5p expression. However, down regulated expression of NORAD combined with up-regulated expression of miR-202-5p partially regulated the EGFR/PI3K/AKT regulatory network. Thus, targeting the interaction between the expressions of NORAD and miR-202-5p, coupled with the EGFR/PI3K/AKT signaling, could serve as a new possible treatment approach to HCC in the future.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Ioannou GN. Epidemiology and risk-stratification of NAFLD-associated HCC. J Hepatol 2021; 75(6): 1476-1484.*
2. *Marrero JA. Hepatocellular carcinoma. Current opinion in gastroenterology 2006; 22(3): 248-253.*
3. *Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A,Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019; 16(10): 589-604.*
4. *Wang H, Huo X, Yang XR, He J, Cheng L, Wang N, Deng X, Jin H, Wang N, Wang C et al. STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4. Mol Cancer 2017; 16(1): 136.*
5. *Fu Y, Wei X, Han Q, Le J, Ma Y, Lin X, Xu Y, Liu N, Wang X, Kong X et al. Identification and characterization of a 25-lncRNA prognostic signature for early recurrence in hepatocellular carcinoma. BMC Cancer 2021; 21(1): 1165.*
6. *Liang C, Pang L, Ke Y, Ji W, Xiong J, Ding R,Ding Y. LncRNA GAS6-AS2 facilitates tumor growth and metastasis of hepatocellular carcinoma by activating the PI3K/AKT/FoxO3a signaling pathway. International journal of clinical and experimental pathology 2019; 12(11): 4011-4023.*
7. *Liu C, Shang Z, Ma Y, Ma J,Song J. HOTAIR/miR-214-3p/FLOT1 axis plays an essential role in the proliferation, migration, and invasion of hepatocellular carcinoma. International journal of clinical and experimental pathology 2019; 12(1): 50-63.*
8. *Jiang L, He Y, Shen G, Ni J, Xia Z, Liu H, Cao Y,Li X. lncRNA HAND2AS1 mediates the downregulation of ROCK2 in hepatocellular carcinoma and inhibits cancer cell proliferation, migration and invasion. Molecular medicine reports 2020.*
9. *Zheng Y, Nie P,Xu S. Long noncoding RNA linc00467 plays an oncogenic role in hepatocellular carcinoma by regulating the miR-18a-5p/NEDD9 axis. Journal of cellular biochemistry 2020.*
10. *Cao X, Zhang G, Li T, Zhou C, Bai L, Zhao J,Tursun T. LINC00657 knockdown suppresses hepatocellular carcinoma progression by sponging miR-424 to regulate PD-L1 expression. Genes Genomics 2020; 42(11): 1361-1368.*
11. *Shen JG, Xu SN,Yin LG. LncRNA NORAD/miR-202-5p regulates the drug resistance of A549/DDP to cisplatin by targeting P-gp. Gen Physiol Biophys 2020; 39(5): 481-489.*
12. *Lin Y, Chen Z, Lin S, Zheng Y, Liu Y, Gao J,Chen S. MiR-202 inhibits the proliferation and invasion of colorectal cancer by targeting UHRF1. Acta Biochim Biophys Sin (Shanghai) 2019; 51(6): 598-606.*
13. *Ke SB, Qiu H, Chen JM, Shi W,Chen YS. MicroRNA-202-5p functions as a tumor suppressor in colorectal carcinoma by directly targeting SMARCC1. Gene 2018; 676: 329-335.*
14. *Zhang L, Xu J, Yang G, Li H,Guo X. miR-202 Inhibits Cell Proliferation, Migration, and Invasion by Targeting Epidermal Growth Factor Receptor in Human Bladder Cancer. Oncol Res 2018; 26(6): 949-957.*
15. *Huang X, Li Y, Zhang J, Xu Y, Tian Y,Ma K. Ganglioside GM3 inhibits hepatoma cell motility via down-regulating activity of EGFR and PI3K/AKT signaling pathway. Journal of cellular biochemistry 2013; 114(7): 1616-1624.*
16. *Wang Y, Yang L, Chen T, Liu X, Guo Y, Zhu Q, Tong X, Yang W, Xu Q, Huang D et al. A novel lncRNA MCM3AP-AS1 promotes the growth of hepatocellular carcinoma by targeting miR-194-5p/FOXA1 axis. Mol Cancer 2019; 18(1): 28.*
17. *He H, Yang H, Liu D,Pei R. LncRNA NORAD promotes thyroid carcinoma progression through targeting miR-202-5p. American journal of translational research 2019; 11(1): 290-299.*
18. *Chen Y, Cao K, Li J, Wang A, Sun L, Tang J, Xiong W, Zhou X, Chen X, Zhou J et al. Overexpression of long non-coding RNA NORAD promotes invasion and migration in malignant melanoma via regulating the*

MIR-205-EGLN2 pathway. Cancer Med 2019; 8(4): 1744-1754.

19. *Meng X, Chen X, Lu P, Ma W, Yue D, Song L,Fan Q. MicroRNA-202 inhibits tumor progression by targeting LAMA1 in esophageal squamous cell carcinoma. Biochem Biophys Res Commun 2016; 473(4): 821-827.*
20. *Hao P, Huang Y, Peng J, Yu J, Guo X, Bao F, Dian Z, An S,Xu TR. IRS4 promotes the progression of non-small cell lung cancer and confers resistance to EGFR-TKI through the activation of PI3K/Akt and Ras-MAPK pathways. Exp Cell Res 2021; 403(2): 112615.*
21. *He J, Huang Z, Han L, Gong Y,Xie C. Mechanisms and management of 3rd-generation EGFR-TKI resistance in advanced non-small cell lung cancer (Review). Int J Oncol 2021; 59(5).*
22. *Garcia-Lezana T, Lopez-Canovas JL,Villanueva A. Signaling pathways in hepatocellular carcinoma. Adv Cancer Res 2021; 149: 63-101.*

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