

Original Article

SNHG5 functions as competitive RNA with miR-23c to regulate HMGB2 expression in hepatocellular carcinoma

Wenchuan Li^{1*}, Yuan Lu^{1*}, Yi Wu³, Zebang Qin³, Qianli Tang¹, Huamei Wei², Jianchu Wang¹, Jian Pu¹

Departments of ¹Hepatobiliary Surgery, ²Pathology, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China; ³Graduate College, Youjiang Medical University for Nationalities, Guangxi, China.
*Equal contributors.

Received October 2, 2019; Accepted May 6, 2020; Epub May 15, 2020; Published May 30, 2020

Abstract: Emerging evidence demonstrated long non-coding RNA (lncRNA) small nucleolar RNA host gene 5 (SNHG5) participates in the tumorigenesis. The aim of this work was to characterize the expression and biology roles of SNHG5 in hepatocellular carcinoma (HCC). Expression level of SNHG5 in HCC cells was analyzed with RT-qPCR. Cell proliferation rate, cell cycle distribution, and cell migration ability was analyzed with cell counting kit-8 assay, flow cytometry, and wound-healing assay, respectively. Targets prediction were performed at LncBase V2.0 and TargetScan. SNHG5 was found elevated expression in HCC cell lines. *In vitro* functional experiments showed knockdown of SNHG5 inhibits cell proliferation and migration, while overexpression of SNHG5 exerted opposite effects. Mechanism studies showed SNHG5 functions as competitive endogenous RNA (ceRNA) for microRNA-23c (miR-23c) to promote high mobility group box 2 (HMGB2) expression. miR-23c was downregulated, while HMGB2 was upregulated in HCC tissues and cells. We revealed SNHG5 could exert an oncogenic role in HCC via regulating miR-23c/HMGB2 axis. Targeting SNHG5 might be a novel therapeutic measure to suppresses HCC progression.

Keywords: SNHG5, miR-23c, HMGB2, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) represents 80-85% of liver cancer cases worldwide [1]. The newly diagnosed cases for liver cancer are 841,080 for worldwide in 2018 [1]. China alone is account for 50% of all liver cancer cases diagnosed each year in a worldwide range [2]. Since the long-term overall survival of HCC remains poor, hence it is essential to explore mechanisms behind HCC progression to help the development of novel therapy methods.

Long non-coding RNAs (lncRNAs) are a class of RNA with length over 200 nucleotides and could function as either tumor suppressor gene or oncogene in cancers [3]. Small nucleolar RNA host gene 5 (SNHG5), 524 bp in length, is a lncRNA that found function as an oncogene in cancers [4-6]. For instance, SNHG5 was elevated expression in breast cancer and promoted cancer progression *in vitro* and *in vivo* [4].

Besides that, SNHG5 was found elevated expression in cisplatin-resistant gastric cancer cells compared with the cisplatin-sensitive cells [5]. Moreover, knockdown of SNHG5 could inhibit gastric cancer proliferation but promote apoptosis through regulating apoptosis associated genes [5]. In HCC, SNHG5 was reported to be upregulated in cancer and significantly correlated with larger tumor size and advanced tumor stages [6]. Functionally, SNHG5 knockdown could inhibit HCC tumor growth *in vitro* and *in vivo*, while SNHG5 overexpression could promote tumor growth [6]. However, we still did not fully understand the biological roles of SNHG5 in HCC and therefore the associated mechanisms of SNHG5 in HCC remain to be deeply explored.

lncRNAs could function as microRNA (miRNA) sponge to regulate target gene expression, which is called competitive RNA (ceRNA) theory [7]. miRNAs are also non-coding RNAs with the

length of 18-25 nucleotides [8]. Previous studies identified several miRNA targets for SNHG5 including miR-154-5p and miR-26a-5p in cancers [4, 6]. miR-23c was revealed decreased expression in HCC and closely correlated with advanced tumor stages and poorer overall survival of cancer patients [9]. Moreover, miR-23c could be regulated by lncRNA KTN1 antisense RNA 1 in HCC to affect HCC progression [10]. Hence, in this work, we explored whether miR-23c might also be a target for SNHG5 in HCC.

High mobility group box 2 (HMGB2) is revealed could influence Warburg effect in breast cancer, and its upregulation could promote cancer malignancy behaviors [11]. Moreover, HMGB2 was found could regulated by miR-329 in melanoma to affect cancer progression [12]. However, its functions and associated mechanisms in HCC remain to be investigated.

In this work, we analyzed the expression level of SNHG5 in HCC cells and investigated the biological functions of SNHG5 in HCC. Moreover, the potential connection of SNHG5 with miR-23c/HMGB2 axis was explored using bioinformatic analysis tool, luciferase activity reporter assay, and rescue experiments.

Materials and methods

Cell culture

Human hepatic cell line LO2, and HCC cells SMMC-7721, and Huh7 were purchased from Cell Bank of Chinese Academy Sciences. These cells were incubated at DMEM contains 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) at a 37°C humidified incubator with 5% CO₂.

Cell transfection

For SNHG5 overexpression, SNHG5 full-length sequence was inserted into pcDNA3.1 to generate pSNHG5 vector. For knockdown of SNHG5 or HMGB2, specific small interfering RNA (si-SNHG5 or si-HMGB2) and corresponding controls (si-NC) were synthesized by GeneChem (Shanghai, China). To overexpress miR-23c, miR-23c mimic and corresponding control (mi-NC) obtained from GeneChem was used. Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Inc.) was used for cell transfection according to manufacturer's instructions.

Real-time quantitative PCR (RT-qPCR)

RNA extracted with Trizol reagent (Invitrogen) from cultured cells were quantified using NanoDrop-1000, and then reverse transcribed into complementary DNA (cDNA) using PrimerScript kit (Takara, Dalian, Liaoning, China). For RT-qPCR analysis, cDNA template, primers, and SYBR Green were mixed and placed into ABI 7500 system (Applied Biosystems, Foster City, CA, USA). U6 snRNA and GAPDH was used as endogenous controls to measure relative gene expression level using 2- $\Delta\Delta$ Ct method. Primers were as follows: SNHG5: 5'-CGAGTAGCCAGTGAAGATAATG-3' (forward) and 5'-CACACAACAGTCAAGTAAACC-3' (reverse); HMGB2: 5'-GGGGAAGAAAAAGGCCCA-3' (forward) and 5'-GCTGACTGCTCAGACCACAT-3' (reverse); GAPDH: 5'-AACGTGTCAGTGGTGGACCTG-3' (forward) and 5'-ATGGCATGGACTGTGGTCAT-3' (reverse); miR-23c: 5'-CCAGAAGGACGTAGAAG-3' (forward) and 5'-CTTCACTGTGATGGGCTC-3' (reverse); U6 snRNA: 5'-CTCGCTTCGGCAGCACA-3' (forward) and 5'-AACGCTTACGAATTTGCGT-3' (reverse). Thermocycling conditions were as follows: 1 cycle at 95°C for 3 min; followed by 40 cycles at 95°C for 10 s, 58°C for 30 s and 72°C for 30 s.

Cell proliferation assay

Cell counting kit-8 (CCK-8, Beyotime, Haimen, Jiangsu, China) assay was used to measure cell proliferation rate according to the manufacturer's instructions. 2,000 cells were seeded into 96-well plate and incubated for indicated time points. Then, CCK-8 reagent was added and further incubation for 4 h. Absorbance was measured at the wavelength of 450 nm.

Flow cytometry

1 × 10⁴ cells were harvested and washed with PBS. Then, cells were fixed with methanol for 30 min, and incubated with propidium iodide (PI, Beyotime) according to the provided protocols. Subsequently, FACS flow cytometer (BD Biosciences, San Jose, CA, USA) with ModFit software was used to analyze cell cycle distribution.

Wound-healing assay

1 × 10⁶ cells were incubated at 24-well plates and cultured to 100% confluence. Wound at

SNHG5/miR-23c/HMGB2 axis contributes to HCC pathogenesis

cell surface was created using pipette tip. Then, PBS was used to remove cell debris. Cell images were captured at 0 or 48 h after wound generation under microscope.

Detection of SNHG5 and miR-23c expression level in HCC tissues using ENCORI

Expression level of SNHG5 and miR-23c in HCC tissues and normal tissues was analyzed using ENCORI.

Detection of HMGB2 expression level in HCC tissues using GEPIA

Expression level of HMGB2 in HCC tissues and normal tissues was analyzed using GEPIA.

Dual-luciferase reporter assay

Bioinformatic analyses tools revealed that SNHG5 and HMGB2 shared the same binding site in miR-23c. Wild-type (wt) fragment of SNHG5 and HMGB2 was inserted into psi-CHECK to generate wt-SNHG5 and wt-HMGB2. Site-direct mutagenesis kit was used to mutant luciferase vectors and named as mt-SNHG5 and st-HMGB2. Cells were transfected with luciferase activity vectors and miRNAs using Lipofectamine 2000 (Invitrogen). 48 h later, relative luciferase activities were measured by Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) using Renilla luciferase activity as internal control.

RIP assay

RIP assays were conducted using EZ-Magna RIP kit (Millipore, Massachusetts, USA) to analyze the interactions of these genes according to manufacturer's instructions. 1×10^7 cells were lysed using RIP lysis buffer, and then incubated with anti-Ago2 or anti-IgG containing magnetic beads. RNA was extracted and subjected to RT-qPCR analysis.

Animal experiments

Animal study protocol was approved by Affiliated Hospital of Youjiang Medical University for Nationalities and performed in accordance with animal care guideline. BALB/c-nu mice were injected with 5×6 cells with sh-SNHG5 or sh-NC stably transfected to develop subcutaneous xenograft tumor. Tumor size was analyzed

every 7 days and then calculate tumor volume. After 4 weeks, mice were sacrificed to obtain tumor tissues.

Haematoxylin and eosin and immunohistochemistry (IHC) analysis

Tumor tissues from animal model were fixed by 4% paraformaldehyde. Tumor tissues were dyed by haematoxylin and eosin (HE) for histological analysis. For IHC analysis of ki-67, the anti-ki-67 antibody was used and performed as previously reported [13].

Statistical analysis

Results were presented as mean \pm SD after analyzing the data from three independent experiments at GraphPad Prism 8.0 (GraphPad Inc., San Diego, CA, USA). One-way analysis of variance or Student's t-test was used for difference analysis in groups. $P < 0.05$ was considered as statistically significant.

Results

LncRNA SNHG5 expression was elevated in HCC

Expression level of lncRNA SNHG5 in HCC tissues and normal tissues analyzed at ENCORI showed SNHG5 expression was higher in HCC tissues than in normal tissues (**Figure 1A**). Similarly, RT-qPCR results showed SNHG5 expression was also significantly higher in HCC cell lines compared with LO2 cell line (**Figure 1B**).

Downregulation of SNHG5 represses HCC cell proliferation and migration in vitro

To explore the roles of SNHG5, si-SNHG5 was introduced into SMMC-7721, which has a relatively high endogenous SNHG5 expression in the cells investigated. Transfection efficacy of si-SNHG5 was analyzed using RT-qPCR (**Figure 2A**). Growth curve calculated by CCK-8 assay showed SNHG5 knockdown decreased cell proliferation rate (**Figure 2B**). Flow cytometry showed cell cycle was arrested at G0/G1 phase after si-SNHG5 transfection (**Figure 2C**). In addition, wound-healing assay showed that cell migration ability was significantly decreased when SNHG5 was downregulated (**Figure 2D**).

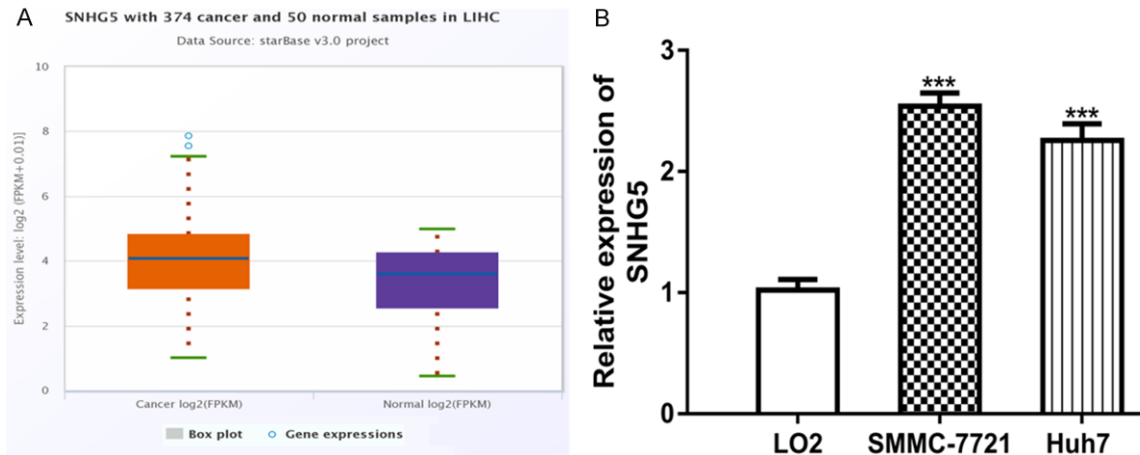


Figure 1. SNHG5 was overexpressed in HCC tissues and cells. A. SNHG5 expression level was higher in HCC tissues than in normal tissues. B. RT-qPCR analysis showed SNHG5 expression level was higher in HCC cells than in normal cell line. SNHG5: small nucleolar RNA host gene 5; HCC: hepatocellular carcinoma; RT-qPCR: Real-time quantitative PCR.

Overexpression of SNHG5 promotes HCC cell proliferation and migration in vitro

Furthermore, we explored roles of SNHG5 through overexpressing SNHG5 in Huh7 cell line. SNHG5 level was dramatically increased by pSNHG5 compared with pcDNA3.1 (Figure 3A). CCK-8 assay and flow cytometry assay revealed that SNHG5 overexpression promote HCC cell proliferation via facilitating cell cycle progression (Figure 3B and 3C). Meanwhile, wound-healing assay showed SNHG5 overexpression significantly promoted migration ability of HCC cell (Figure 3D).

SNHG5 serves as miR-23c sponge to regulate HMGB2 expression

To understand the mechanism underlying role of SNHG5 in tumorigenesis, we investigated miRNA targets of SNHG5. As presented in Figure 4A, miR-23c contains a binding site for SNHG5. Figure 4B revealed miR-23c was decreased expression in HCC tissues compared with normal tissues. Co-transfection of luciferase vectors and miRNAs showed miR-23c mimic could reduce relative luciferase activity in HCC cells transfected with wt-SNHG5 (Figure 4C). Moreover, knockdown of SNHG5 increased miR-23c expression in HCC cells (Figure 4D). As miRNA regulate tumor progression through regulating specific targets, we searched the targets for miR-23c using TargetScan. We found 3'-UTR of HMGB2 contains a binding site for miR-23c (Figure 4E). HMGB2 expression level

was found higher in HCC tissues than in normal tissues (Figure 4F). Luciferase activity reporter assay showed transfection of miR-23c mimic decreased luciferase activity of cells with wt-HMGB2 transfection (Figure 4G). Moreover, we found knockdown of SNHG5 decreased HMGB2 expression in HCC cell (Figure 4H). In addition, increased miR-23c expression suppressed HMGB2 expression in HCC cell (Figure 4I). RIP assay showed SNHG5, miR-23c, and HMGB2 were co-enriched (Figure 4J).

SNHG5 promotes HCC cell growth via miR-23c/HMGB2

We then investigated whether SNHG5 regulates HCC cell behaviors via miR-23c/HMGB2 axis. Results of CCK-8 assay showed miR-23c overexpression inhibits HCC cell proliferation, and partially abolished the effects of pSNHG5 (Figure 5A). Consistent with CCK-8 assay results, flow cytometry assay and wound-healing assay showed the effects of SNHG5 on HCC cells could be abrogated by miR-23c overexpression (Figure 5B and 5C). After that, we investigated the involvement of HMGB2 in the SNHG5 mediated stimulation effects of HCC cells. Results showed the effects of pSNHG5 on HCC cell behaviors could be reversed by si-HMGB2 (Figure 5D-F).

SNHG5 regulates HCC tumor growth in vivo

The analysis of tumor weight and tumor volume after SNHG5 knockdown showed that tumor

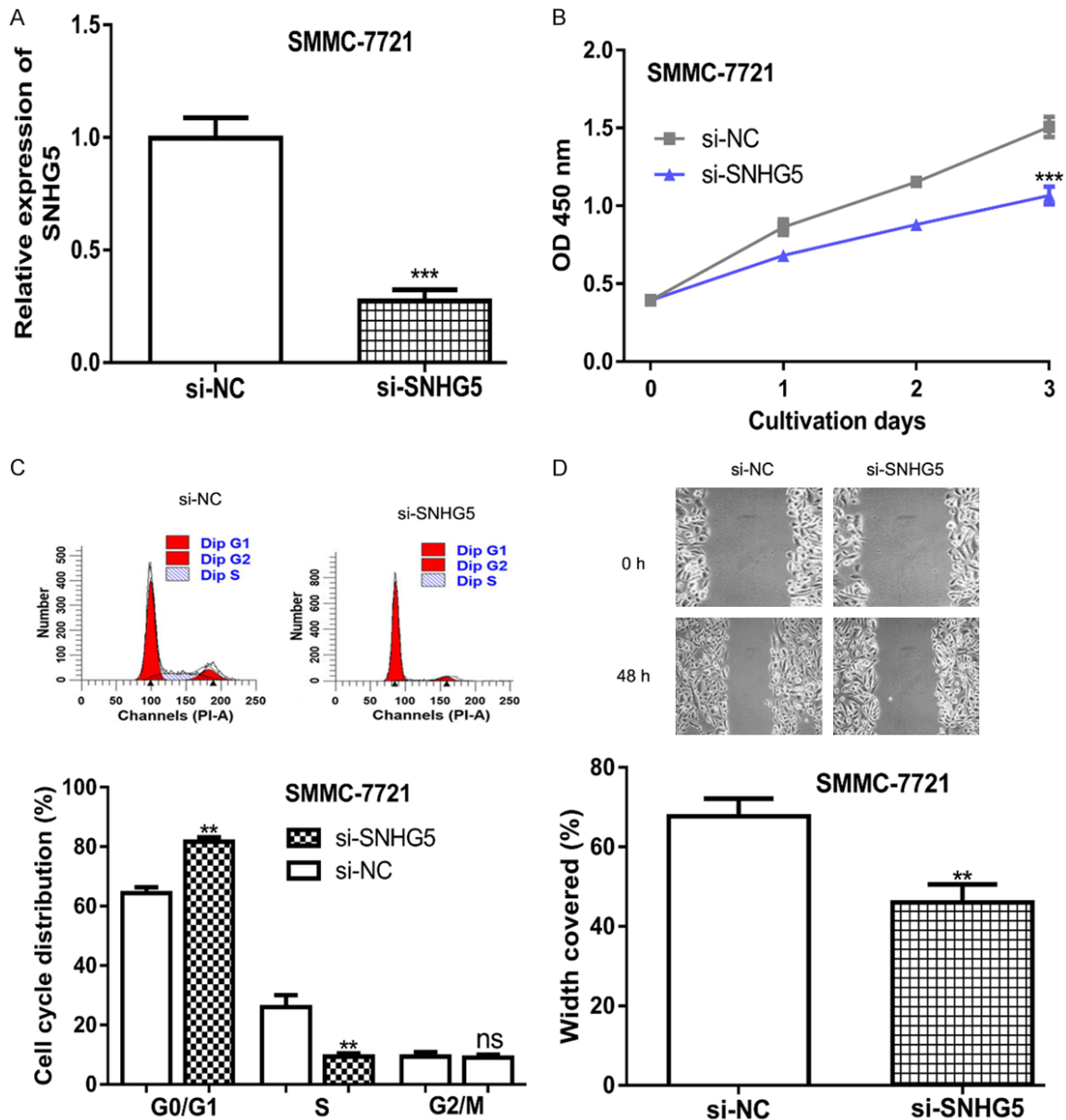


Figure 2. Downregulation of SNHG5 inhibits proliferation and migration in HCC line. A. RT-qPCR analysis showed SNHG5 expression level was decreased in HCC cell following si-SNHG5 transfection. B. CCK-8 assay showed silencing of SNHG5 inhibited the proliferation rate of HCC cells. C. SNHG5 knockdown caused HCC cell cycle arrested at G0/G1 phase. D. Wound-healing assay showed SNHG5 knockdown reduced migration ability of HCC cells. SNHG5: small nucleolar RNA host gene 5; HCC: hepatocellular carcinoma; RT-qPCR: Real-time quantitative PCR; CCK-8: cell counting kit-8; si-SNHG5: small interfering RNA targeting SNHG5; si-NC: negative control siRNA.

weight and tumor volume was significantly reduced by SNHG5 knockdown compared with sh-NC (Figure 6A and 6B). Results obtained from HE and IHC staining of ki-67 validated the changes in tumor formation (Figure 6C).

Discussion

In recent years, immune targeted therapy methods for HCC have been gradually developed,

which has helped us to improve the survival quality of cancer patients [14]. Dysregulation of lncRNAs was found involve in almost all cellular behaviors, and affect almost every hallmarks of cancer [15]. LncRNA HOXA11-AS was found elevated expression in HCC, and correlated with poorer overall survival of cancer patients [16]. In addition, HOXA11-AS was found could promote HCC cell migration and invasion through regulating miR-124/EZH2 axis [16].

SNHG5/miR-23c/HMGB2 axis contributes to HCC pathogenesis

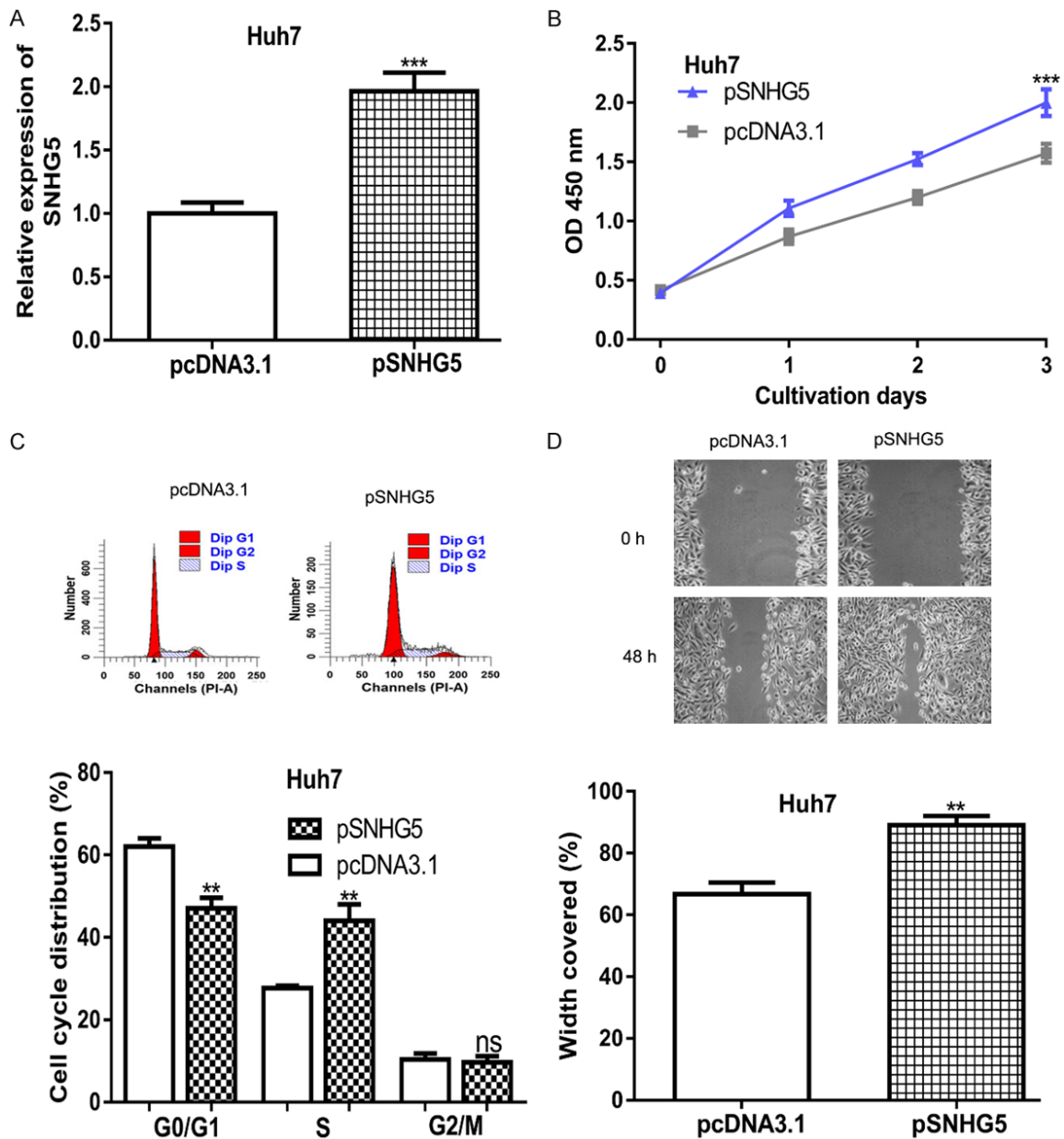


Figure 3. Overexpression of SNHG5 promotes proliferation and migration in HCC line. **A.** RT-qPCR analysis showed SNHG5 expression was increased in HCC cell following pSNHG5 transfection. **B.** CCK-8 assay showed overexpression of SNHG5 promoted proliferation rate of HCC cells. **C.** SNHG5 overexpression promoted HCC cell cycle progression. **D.** Wound-healing assay showed SNHG5 overexpression promoted migration ability of HCC cells. SNHG5: small nucleolar RNA host gene 5; HCC: hepatocellular carcinoma; RT-qPCR: Real-time quantitative PCR; CCK-8: cell counting kit-8.

SNHG5 has been reported to function as an oncogene in cancers including breast cancer and gastric cancer [4, 5], however, the roles of SNHG5 in HCC require further investigations. In line with these studies, we also revealed the upregulation status of SNHG5 in HCC. Uncontrolled cell proliferation and migration are the hallmarks of human cancers [15]. Here,

we showed SNHG5 overexpression could promote HCC cell proliferation and migration *in vitro*. Moreover, knockdown of SNHG5 inhibits HCC cell proliferation and lead to cell cycle arrest at G0/G1 phase. The regulatory mechanism, ceRNA theory, related to lncRNA has been reported in recent years. Hence, we are interested to investigate whether SNHG5 func-

SNHG5/miR-23c/HMGB2 axis contributes to HCC pathogenesis

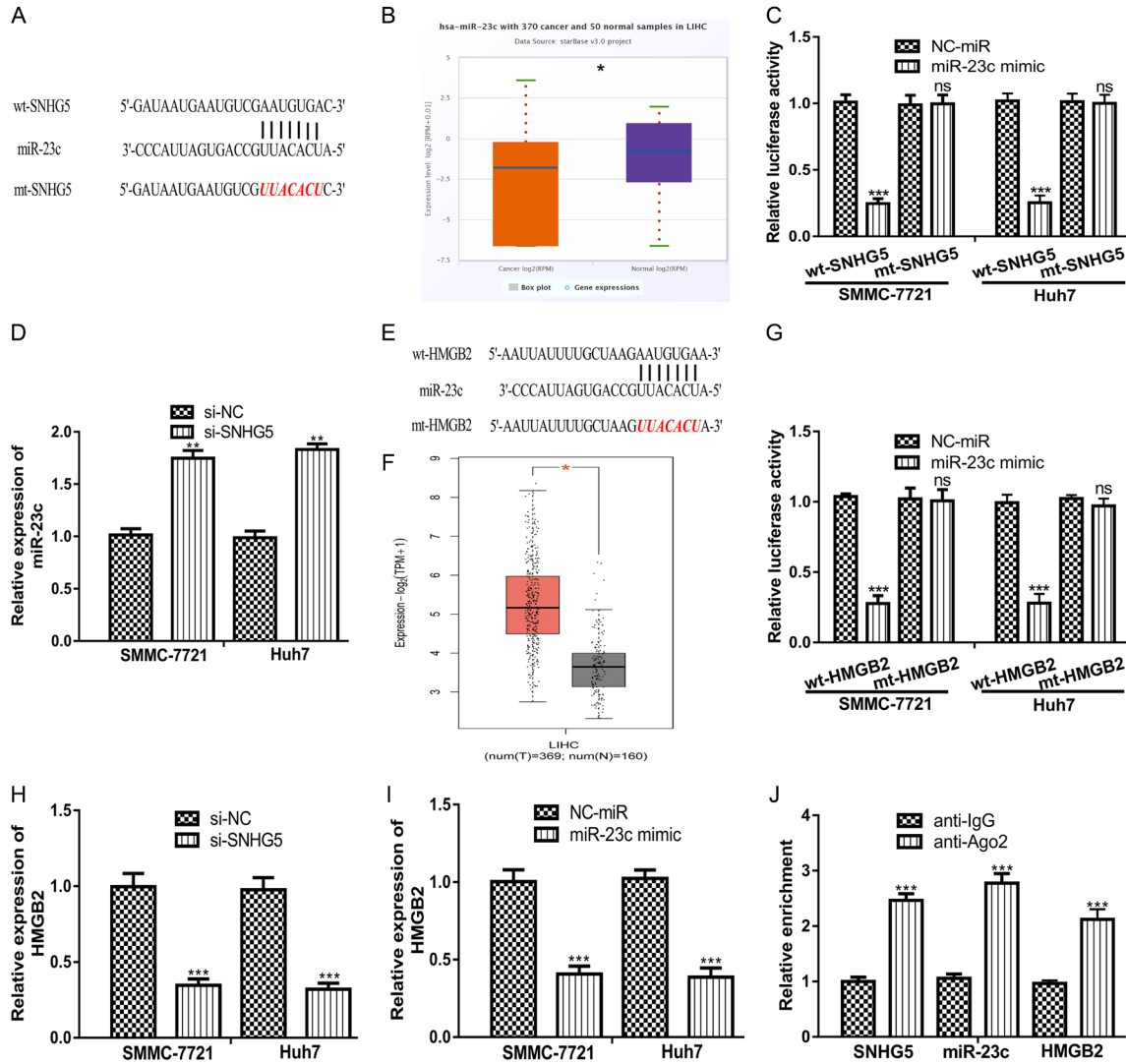


Figure 4. SNHG5 acts as a competing endogenous RNA to regulate HMGB2 expression by binding with miR-23c. A. Binding module between SNHG5 and miR-23c. B. miR-23c expression was decreased in HCC tissues compared with normal tissues. C. Luciferase reporter assay validated the interaction between SNHG5 and miR-23c in HCC. D. SNHG5 knockdown increased miR-23c expression level in HCC cells. E. Binding module between HMGB2 and miR-23c. F. HMGB2 expression level was increased in HCC tissues compared with normal tissues. G. Luciferase reporter assay validated the interaction between HMGB2 and miR-23c. H. SNHG5 knockdown decreased HMGB2 expression in HCC cells. I. miR-23c overexpression decreased HMGB2 expression in HCC cells. J. Co-enrichment of SNHG5, miR-23c, and HMGB2 in HCC cell. SNHG5: small nucleolar RNA host gene 5; HCC: hepatocellular carcinoma; miR-23c: microRNA-23c; HMGB2: high mobility group box 2; NC-miR: negative control miRNA; wt: wild type; mt: mutant.

tions as a ceRNA in HCC. We showed miR-23c expression level was significantly decreased in HCC tissues. Moreover, SNHG5 may direct interact with miR-23c to regulate proliferation and migration of HCC cells. The functions of miR-23c in cancers have been widely investigated in recent years. miR-23c was a miRNA that reported could be regulated by lncRNA KTN1 antisense RNA 1 to inhibit HCC tumor growth [17, 18]. Here, we showed SNHG5 could regulate miR-23c, and knockdown of SNHG5 inhibits HMGB2 expression via serving as

sponge for miR-23c. Hence, our results showed SNHG5/miR-23c/HMGB2 axis in regulating the proliferation and migration of HCC cells, indicating SNHG5 may also function as oncogenic lncRNA in HCC. Although we achieved some progresses on understanding the roles of SNHG5 in HCC, it is far from enough evidence to support the clinical application of SNHG5 in HCC. Therefore, in the future, researches by recruiting HCC patients into study to explore the significance of SNHG5 in HCC are urgently needed.

SNHG5/miR-23c/HMGB2 axis contributes to HCC pathogenesis

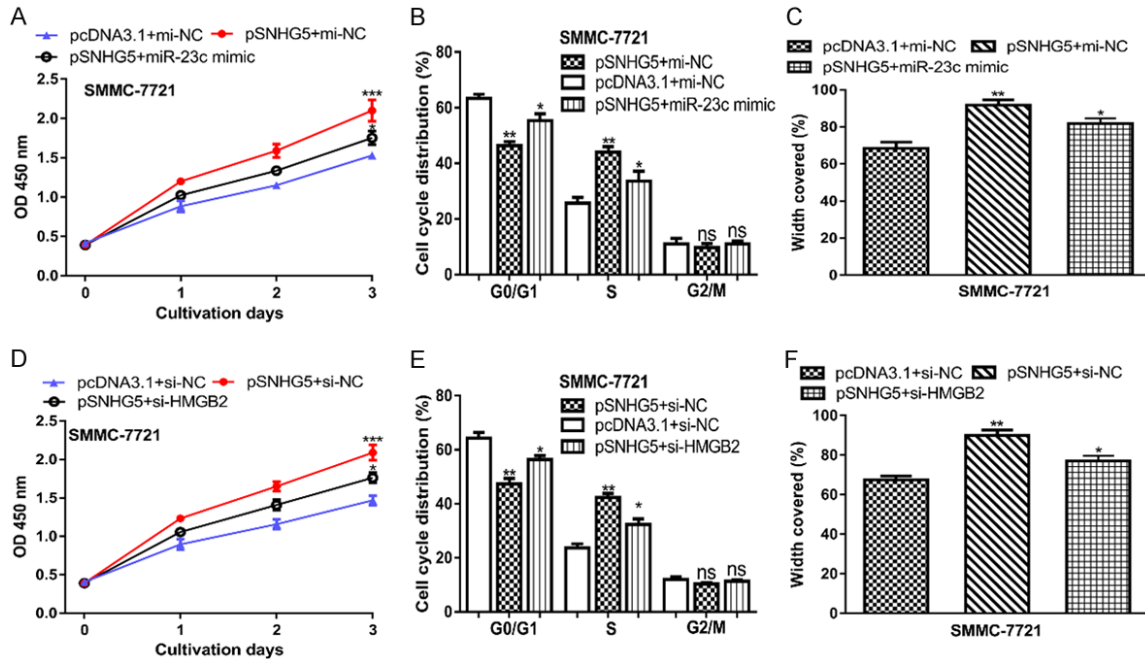


Figure 5. SNHG5 promotes HCC cell growth by inhibiting miR-23c/HMGB2 axis. A-C. CCK-8 assay, flow cytometry assay, and wound-healing assays demonstrated SNHG5 reversed the inhibitory effects of miR-23c on HCC cell behaviors. D-F. CCK-8 assay, flow cytometry assay, and wound-healing assays demonstrated HMGB2 overexpression reversed the suppression effects of SNHG5 knockdown on HCC cell behaviors. SNHG5: small nucleolar RNA host gene 5; HCC: hepatocellular carcinoma; miR-23c: microRNA-23c; HMGB2: high mobility group box 2; si-SNHG5: small interfering RNA targeting SNHG5; si-NC: negative control siRNA; mi-NC: negative control miRNA; CCK-8: cell counting kit-8.

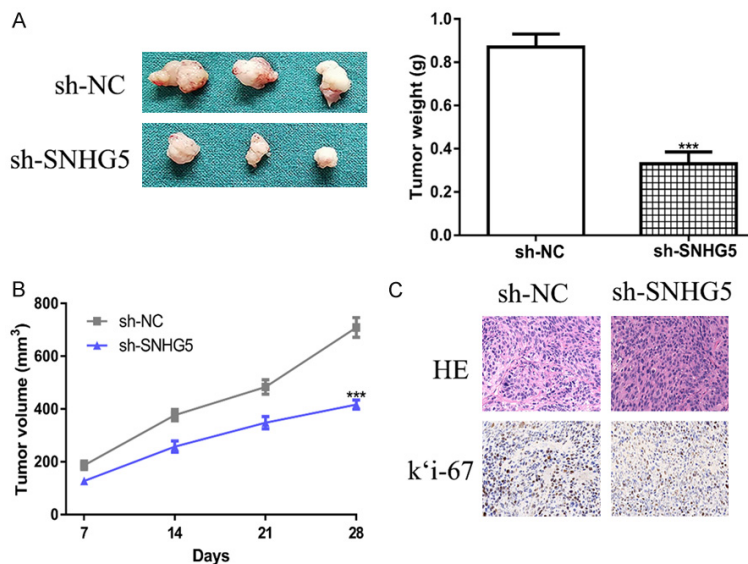


Figure 6. SNHG5 regulates HCC tumor growth in vivo. A. The weight of tumors from animal model with sh-SNHG5 or sh-NC transfection. B. The tumor growth curve of tumors from animal model with sh-SNHG5 or sh-NC transfection. C. HE and IHC of ki-67 in tumor tissues. SNHG5: small nucleolar RNA host gene 5; HCC: hepatocellular carcinoma; HE: Haematoxylin and eosin; IHC: Immunohistochemistry; sh-SNHG5: short hairpin RNA targeting SNHG5; sh-NC: negative control shRNA.

Conclusion

In summary, our study indicated SNHG5 was elevated expression in both HCC tissues and cell lines, indicating SNHG5 functions as an oncogenic lncRNA to promote tumorigenesis by regulating HMGB2 through competitively binding miR-23c. This work provided evidence that SNHG5 may be a novel therapeutic target for HCC.

Acknowledgements

This work was supported by Science and Technology Project (AD17129025).

Disclosure of conflict of interest

None.

SNHG5/miR-23c/HMGB2 axis contributes to HCC pathogenesis

Address correspondence to: Jian Pu, Department of Hepatobiliary Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China. E-mail: yj_pujian@163.com

References

- [1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; 68: 394-424.
- [2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; 66: 115-132.
- [3] Bach DH and Lee SK. Long noncoding RNAs in cancer cells. *Cancer Lett* 2018; 419: 152-166.
- [4] Chi JR, Yu ZH, Liu BW, Zhang D, Ge J, Yu Y and Cao XC. SNHG5 promotes breast cancer proliferation by sponging the miR-154-5p/PCNA axis. *Mol Ther Nucleic Acids* 2019; 17: 138-149.
- [5] Li M, Zhang YY, Shang J and Xu YD. LncRNA SNHG5 promotes cisplatin resistance in gastric cancer via inhibiting cell apoptosis. *Eur Rev Med Pharmacol Sci* 2019; 23: 4185-4191.
- [6] Li Y, Guo D, Zhao Y, Ren M, Lu G, Wang Y, Zhang J, Mi C, He S and Lu X. Long non-coding RNA SNHG5 promotes human hepatocellular carcinoma progression by regulating miR-26a-5p/GSK3 β signal pathway. *Cell Death Dis* 2018; 9: 888.
- [7] Qi X, Zhang DH, Wu N, Xiao JH, Wang X and Ma W. ceRNA in cancer: possible functions and clinical implications. *J Med Genet* 2015; 52: 710-718.
- [8] Vishnoi A and Rani S. MiRNA biogenesis and regulation of diseases: an overview. *Methods Mol Biol* 2017; 1509: 1-10.
- [9] Zhang L, Wang Y, Wang L, Yin G, Li W, Xian Y, Yang W and Liu Q. miR-23c suppresses tumor growth of human hepatocellular carcinoma by attenuating ERBB2IP. *Biomed Pharmacother* 2018; 107: 424-432.
- [10] Zhang L, Wang L, Wang Y, Chen T, Liu R, Yang W, Liu Q and Tu K. LncRNA KTN1-AS1 promotes tumor growth of hepatocellular carcinoma by targeting miR-23c/ERBB2IP axis. *Biomed Pharmacother* 2019; 109: 1140-1147.
- [11] Fu D, Li J, Wei J, Zhang Z, Luo Y, Tan H and Ren C. HMGB2 is associated with malignancy and regulates Warburg effect by targeting LDHB and FBP1 in breast cancer. *Cell Commun Signal* 2018; 16: 8.
- [12] Mo Y, Fang RH, Wu J, Si Y, Jia SQ, Li Q, Bai JZ, She XN and Wang JQ. MicroRNA-329 upregulation impairs the HMGB2/ β -catenin pathway and regulates cell biological behaviors in melanoma. *J Cell Physiol* 2019; 234: 23518-23527.
- [13] Zhang J, Wang G, Chu SJ, Zhu JS, Zhang R, Lu WW, Xia LQ, Lu YM, Da W and Sun Q. Loss of large tumor suppressor 1 promotes growth and metastasis of gastric cancer cells through upregulation of the YAP signaling. *Oncotarget* 2016; 7: 16180-16193.
- [14] Neureiter D, Stintzing S, Kiesslich T and Ocker M. Hepatocellular carcinoma: therapeutic advances in signaling, epigenetic and immune targets. *World J Gastroenterol* 2019; 25: 3136-3150.
- [15] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-674.
- [16] Zhang WL, Zhao YN, Shi ZZ, Gu GY, Cong D, Wei C and Bai YS. HOXA11-AS promotes the migration and invasion of hepatocellular carcinoma cells by inhibiting miR-124 expression by binding to EZH2. *Hum Cell* 2019; 32: 504-514.
- [17] Zhang L, Wang Y, Wang L, Yin G, Li W, Xian Y, Yang W and Liu Q. miR-23c suppresses tumor growth of human hepatocellular carcinoma by attenuating ERBB2IP. *Biomed Pharmacother* 2018; 107: 424-432.
- [18] Zhang L, Wang L, Wang Y, Chen T, Liu R, Yang W, Liu Q and Tu K. LncRNA KTN1-AS1 promotes tumor growth of hepatocellular carcinoma by targeting miR-23c/ERBB2IP axis. *Biomed Pharmacother* 2019; 109: 1140-1147.