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Upregulation of microRNA-483-5p Advances Non-small Cell Lung Cancer (NSCLC) Progression via Stifling HIPK2 Expression

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Abstract

Background: Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer and has a high incidence and mortality rate. The combination of radiotherapy and chemotherapy is used widely to treat locally advanced NSCLC, but the clinical efficacy is limited. MiRNA-483-5p has been connected to the improvement of an assortment of malignancies. Notwithstanding, its capacity in NSCLC stays obscure.

Methods: Here we utilized benefit- or loss-of-miRNA-483-5p expression to investigate the effect of miRNA-483-5p on NSCLC.

Results: The results showed that MiRNA-483-5p is entirely up-regulated in NSCLC tissues and cell lines. MiRNA-483-5p inhibitor blocked cell viability, proliferation, migration, invasion but promoted apoptosis, suggesting miRNA-483-5p acts as an oncogene in NSCLC. TargetScan predicted that HIPK2 was an objective gene of miRNA-483-5p. Then, luciferase reporter assay further confirmed that miRNA-483-5p specifically attacked HIPK2's 3'UTR, suggesting the targeted relationship between miRNA-483-5p and HIPK2. Moreover, HIPK2 acted as a redox signal modulator and was associated with a variety of malignant tumors. The current examination affirmed the low HIPK2 expression in the NSCLC tissues and cell lines. Moreover, overexpression of HIPK2 inhibited NSCLC cell viability, proliferation, migration, invasion, but enhanced apoptosis. More importantly, co-transfection with HIPK2 and miRNA-483-5p reversed these effects, suggesting that miRNA-483-5p facilitated tumor progression by inhibiting HIPK2.

Conclusions: Hence, our findings indicated that miRNA-483-5p might be a promising remedial target in NSCLC and give major premise to clinical therapeutics.

Introduction

Non-small-cell lung cancer (NSCLC) which is the most common form of lung cancer, is also the world's most well-known and deadly tumor, as well as the most lethal cancer in China, posing a serious health threat [1–3]. Albeit incredible advancement has been acquired in early identification and treatment procedures in the course of recent many years, the endurance rate actually remains low [4]. Subsequently, further examination on the sub-atomic systems is fundamental for NSCLC early detection and treatment strategies.

MicroRNAs (miRNAs) are 18–22 nucleotide noncoding RNAs that bind to 3' untranslated region (3'UTR) of mRNA (the objective courier RNA) to regulate target gene expression [5]. Cell proliferation, apoptosis, differentiation, and growth are all regulated by miRNAs [6, 7]. In a growing number of studies, miRNA expression has been linked to the NSCLC progression [8–10]. Deeper understanding of molecular mechanisms on NSCLC is significant to early detection and treatment strategies.

MiRNA-483-5p has been identified as a possible osteoarthritis biomarker that is involved in metabolic cycles [11]. Tumorigenesis is triggered by abnormal miRNA-483-5p expression in a variety of human cancers [12]. MiRNA-483-5p can regulate mitochondrial splitting in tongue squamous cell carcinoma, controlling drug sensitivity along with its target gene, *FIS1* [13]. Nonetheless, the impact and mechanism of miRNA-483-5p on the NSCLC still actually stay hazy. Homeodomain-interacting protein kinase 2 (HIPK2) was reported to be associated with various malignant tumors, including lung cancer [14]. We used bioinformatic algorithms (TargetScan) to search for putative miRNA-483-5p targets in this study, and found that HIPK2 was a possible miRNA-483-5p target. The miRNA-483-5p expression was higher in NSCLC tissues and cells, according to the findings. By focusing on HIPK2, MiRNA-483-5p was able to regulate NSCLC cell migration, apoptosis, proliferation, and invasion.

Materials And Methods

Experiments on human tissue

Patients with NSCLC were recruited from Affiliated Wujiang Hospital of Nantong University between November 2018 and December 2020. All tolerant analyze of NSCLC had been affirmed dependent on obsessive measure, and none of the patients got any overall malignant growth treatment. Educated assent was acquired from all patients. NSCLC tumor tissues and adjacent non-tumor tissues were obtained from each participant during surgical section with written informed consent. The present study was approved by the Ethics Committee of Affiliated Wujiang Hospital Nantong University following the Declaration of Helsinki.

Cell culture and transfection

The Chinese Academy of Science Cell Bank provided the H358, H292, A549, and H1299 NSCLC cell lines, as well as the human bronchial epithelioid cells 16HBE (Shanghai, China). At 37°C and 5% CO_2, cells were cultured in RPMI-1640 medium (Hyclone, South Logan, UT, USA) supplemented with 10% FBS (fetal cow serum, Biowest, Barcelona, Catalonia, Spain). GenePharma (Shanghai, China) designed and supported Si-HIPK2 and its negative control Si-NC, miRNA-483-5p inhibitors, inhibitors as well as their negative controls (inhibitor NC and mimic NC). pcDNA 3.1 HIPK2 was created by cloning human cDNA encoding HIPK2 into the pcDNA3.1 vector using BamH/XhoI sites. Forward: 5'-GGTATTTGACTTCAGTCAA-3'; Reverse: 5'-GGCTGAGACTAGTACAGTT-3'. To transfect cells with the recombinant plasmids, Lipofectamine 2000 was used (Invitrogen, Carlsbad, CA, USA). Following 24 to 48 h, transfected cells were gathered and applied in after examinations.

qRT-PCR

To measure miRNA-483-5p expression, we used a miRcute miRNA Isolation unit (Tiangen, Shanghai, China) to extract complete RNA and a One Step PrimeScript miRNA cDNA Synthesis unit to reverse translate for cDNA (Takara Biotechnology Co., Ltd.). We used the TRIzol Kit (Invitrogen, Carlsbad, CA, USA) to extract complete RNA and then interpreted the cDNA with M-MLV reverse transcriptase to detect HIPK2 mRNA expression (Promega, Madison, WI, USA). The SYBR ExScript RT-qPCR Kit (Takara, Dalian, China) was used in the ABI 7500 Real-Time PCR system to perform qRT-PCR (Applied Biosystems; Thermo Fisher

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10 µL SYBR Premix EX Taq, and 7 µL ddH2O. The following was the PCR protocol: 94°C for 5 min, then 35 intervals of 94°C for 10 s and 63°C for 30 s. The same Cq (ΔΔCq) methodology was used to evaluate all overlay adjustments, with GAPHD and U6 used for standardization. The primers sequences were as follows: miRNA-483-5p (F): 5′-TCAACGGGACAGACAAAGAT-3′, miRNA-483-5p (R): 5′-CTCAGGATGGAGCAGAGGG-3′; U6 (F): 5′-TATGGCTCCTTTCACCTG-3′, U6 (R): 5′-CCTGGCAGACAGTCAGAA-3′; HIPK2 (F): 5′-GCTTCCAGCACAAGAACCACAC-3′, HIPK2 (R): 5′-GCAATGACACAACCAAGGGACC-3′; GAPDH (F): 5′-CTTCACCACCATGGAGAAGGC-3′, GAPDH (R): 5′-GGCATGGACTGTGGTCATGAG-3′.

Western blotting

All out protein from cells was lysed with RIPA buffer (Invitrogen, USA), and protein fixation was inspected by BCA kit (Beyotime, Haimen, China). A 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel was used to isolate 50 g of all out proteins per route, which were then smeared onto PVDF membranes. Before primary antibody incubation, PDVF membranes were blocked in 5% skimmed milk at 37℃. Key antibodies for HIPK2 (1:2000, ab108543), Bax (1:1500, ab32503), Bcl-2 (1:1000, ab32124), Cleaved-caspase-3 (1:500, ab2302), Cleaved-caspase-9 (1:1000, ab2324), MMP-2 (1:1000, ab92536), MMP-9 (1:1000, ab73734) and GAPDH (1:2500, ab9485) were added on the PDVF membranes and incubated at 4℃ overnight. The next day, secondary antibodies (1:1000, ab96899) were hatched to the layers. All antibodies were purchased from Abcam in Cambridge, Massachusetts, Using an ECL kit, the groups were tested visually (Beyotime Institute of Biotechnology, Beijing, China), and Image J software version 1.45 was used to calculate the dim worth of the groups (NIH, Bethesda, MD, USA).

Assay for cell proliferation

To identify cell proliferation, the producers used cell counting kit-8 (CCK-8), colony formation and EdU examinations. For CCK-8 assay, Cells were inoculated in a 96-well plate with the density of 1×10^4/well. At 0, 24, 48 and 72 h, 10 µL CCK-8 reagent (Takara, Japan) was added to each plate. After incubation for another 2 h, the absorbance at 450nm was measured with a microplate analyzer. For colony formation assay, cells were grown in a 6-well plate at a thickness of 1.1×10^3/well for 14 d while colony formation test. The clones were fixed in methanol and stained with crystal violet (0.1 percent). For EdU examine, cells (1×10^5) were kept up in 6-well plate. After 48 h, EdU (100µL) was added for 2.5 h. Subsequently, cells were treated with paraformaldehyde (4%) and then added Triton X-100 (0.5%). For investigation, EdU-positive cells were examined utilizing an Olympus fluorescence microscopy at × 200 magnification (Olympus, Tokyo, Japan). For the Ki67 staining, tumor tissues from nude mice xenograft model were treated as for IHC. Rabbit anti-Ki67 antibodies (1:300, ab15580) were used for staining, and Ki67 positive cells were scored at ×200 magnification.

Cell apoptosis examine

The FITC-Annexin V/PI Apoptosis Detection unit was used to classify cell apoptosis using flow cytometry (BD Pharmingen, San Diego, CA, USA). 1.1×10^5 cells were stained with 4.5 mL propidium iodide and 4.5 mL Annexin V-FITC, followed by Flow cytometer fluorescence assurance (Beckman, Miami, FL, USA). All investigations were estimated in 3 times. TdT-mediated dUTP nick end labeling (TUNEL) assay was performed to detect the apoptosis level in the tumor tissues from nude mice xenograft model following the manufacturer's instruction (Promega). Tunel positive cells were scored at ×200 magnification.

Scratch assay

Cells were grown using 6-well plates. After reaching 80% confluence, cells were put in the divider and rinsed twice in 1×PBS to eliminate coasting cells. To make the wounds, sterile pipette tips were used. Then, cells were rinsed twice in 1×PBS before being placed in a culture plate with 2 mL RPMI-1640 culture medium (10% FBS). Photos were taken at 48 h after the injury was formed.

Assays of transwell migration and matrigel invasion

Transwell migration and matrigel invasion assays were carried out utilizing Transwell chambers as indicated by the producer's guidelines. A matrigel grid was covered in the transwell membrane and utilized for the cell invasion test. In short, 1×10^5 cells/well in DMEM (100L,0.6%FBS) were put in the upper Transwell chamber (Corning Incorporated, NY, USA) that had been pre-coated with matrigel (Growth factor reduced, BD Biosciences, MD, USA). DMEM was loaded into the base chambers (20 % FBS). In the upper Transwell chamber, 1×10^5 cells/well in DMEM (100L, 0.5 % FBS) were placed for the migration test (Corning Incorporated, NY, USA).The base chambers were loaded up with DMEM (20% FBS). After 24 h, the cells were fixed and stained in both the Transwell migration and matrigel invasion examine. Haphazardly chose fields were checked under a reversed magnifying lens (200 × magnification, Carl ZEISS, Jena, German) and a normal worth was utilized as the quantity of attacked cells.

Luciferase reporter assays

Amplify and clone the HIPK2-3′-UTR into the pGL3-basic vector to construct pGL3-HIPK2-WT-3′UTR. Quik Change Site-Directed Mutagenesis Kit (AgilentTechnologies) was used to construct a mutant version (pGL3-HIPIK2-Mut-3′UTR). DNA sequencing was used to validate all plasmids. Using Lipofectamine 2000, the A549 cells were co-transfected with miRNA-483-5p mimic or mimic NC and HIPK2-WT-3′UTR or HIPIK2-Mut-3′UTRin 24-well plates (Invitrogen). After 48 h, luciferase activity was calculated using the Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) and normalized with *Renilla* luciferase.

Tumor nude mice xenograft model

5-weeks BALB/c nude mice (19–21 g) were purchased and acclimated to standard temperature, relative humidity, and light conditions for several weeks from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Cells steadily transfected with recombinant plasmid were injected subcutaneously into the mice (n = 8 per group). Per week, the tumor size was calculated using calipers [(length width2)/2]. Mice were fixed using the plate after being anesthetized with pentobarbital sodium (1%, 35 mg/kg, Dainippon Sumitomo Pharma) at the 5th week. After tumors were taken out gauged and broke down, mice were sacrificed by bloodletting. All systems and creature tests were endorsed by Affiliated Wujiang Hospital of Nantong University Ethics Committee (No.

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Statistical analysis

All the results are recorded as means and standard deviations. GraphPad program 9.0 was used to conduct all factual inquiries. Student's t-test or a one-way analysis of variance (ANOVA) test followed by Tukey's multiple comparison test was used to assess the differences between two or at least three groups. Statistical significance defined as $p<0.05$.

Results

In NSCLC cell lines and tissues, MiRNA-483-5p is upregulated

To distinguish qualified microRNAs in NSCLC, we examined microarray expression profiles. 3 NSCLC microarray datasets were selected and analyzed for consistently aberrant microRNAs between NSCLC and normal tissues. We discovered that miRNA-483-5p was significantly overexpressed in NSCLC patients (Fig. 1A). Furthermore, we investigated the expression pattern of miRNA-483-5p in NSCLC to learn more about its effect on lung cancer cells. A qRT-PCR assay was used to determine the miRNA-483-5p expression in 26 human NSCLC lung tissues and H358, H292, A549, and H1299 cell lines. MiRNA-483-5p expression in NSCLC lung tissues was significantly higher than normal lung tissues (P□0.05, Fig. 1B) and lung cancer cell lines (P□0.01, Fig. 1C). More importantly, the expression of miRNA-483-5p was incraesed most prominently in A549 cells compared with 16HBE among all lung cancer cell lines, hence, A549 was chosen for following experiments. MiRNA-483-5p was found to be up-regulated in NSCLC, according to the findings. To investigate the function of miRNA-483-5p in cell proliferation, we overexpressed and down-regulated miRNA-483-5p with miRNA-483-5p mimic or inhibitors. qRT-PCR research revealed that transfection of miRNA-483-5p inhibitors effectively down-regulate miRNA-483-5p and miRNA-483-5p mimic up-regulate miRNA-483-5p effectively (Fig. 1D).

In vitro, MiRNA-483-5p promotes NSCLC cell proliferation thus inhibiting apoptosis

The CCK-8 test revealed that the A549 cells proliferation was reduced in the miRNA-483-5p inhibitor group while promoted in miRNA-483-5p mimic group. (Fig. 2A). Consistent with CCK-8 assay, colony formation and EdU assays obtained similar results (Fig. 2B, C). We also used flow cytometry to estimate cell apoptosis. The results indicated that A549 cells in the miRNA-483-5p mimic-transfected group had a lower apoptosis ability while the apoptosis was significantly enhanced in miRNA-483-5p inhibitor group. (Fig. 2D). We also applied western blotting to detect apoptosis-related protein level, finding that Bax (pro-apoptosis protein) and Cleaved-caspase-3/9 was up-regulated and anti-apoptosis proteins Bcl-2 were down-regulated in miRNA-483-5p inhibitor group; however, the variance of Bax, Bcl-2 and Cleaved-caspase-3/9 presented the opposite trends in miRNA-483-5p mimic group compared with miRNA-483-5p inhibitor group (Fig. 2E). These discoveries showed that miRNA-483-5p stimulated A549 cell proliferation thus suppressing apoptosis.

In vivo, MiRNA-483-5p promotes NSCLC tumor growth

Given the ability of miRNA-483-5p in vitro, we investigated whether miRNA-483-5p dysfunction could promote tumor growth in vivo. The mice were sacrificed and the tumor was resected five weeks after injection. As a result, tumors from miRNA-483-5p mimic group developed significantly faster than tumors from other classes. The tumor was clearly larger and heavier than the other gatherings, with a maximum tumor volume of 3039.565 mm^3 (Fig. 3A, B). On the contrary, the tumor volume was the lightest in miRNA-483-5p inhibitor group. Additionally, immunohistochemical analysis showed that the proliferation related protein ki67 was up-regulated in miRNA-483-5p mimic group, while expression was inhibited in the inhibitor group (Fig. 3C). Besides, result of tunel assay indicated that the apoptosis level was up-regulated in inhibitor group (Fig. 3D). These outcomes inferred that miRNA-483-5p may promote tumor progression.

In vitro, MiRNA-483-5p promotes NSCLC cell invasion and migration

Scratch and transwell assays were used to investigate the impact of miRNA-483-5p on cell migration and invasion. Scratch tests revealed that the wound closure was significantly decreased in the inhibitor group while increased in mimic group (P□0.05), as shown in Fig. 4A. Transwell analysis revealed that in the control group, more cells were moved or attacked to the lower medium than in the miRNA-483-5p mimic group; while miRNA-483-5p inhibitor group displayed the opposite results (Fig. 4B, C). Matrix metalloproteinases (MMP2/9), which are involved in cancer cell migration and invasion, is classified using western blotting [15, 16]. The expression of MMP-2 and MMP-9 were reduced in the miRNA-483-5p inhibitor and increased in the mimic group (Fig. 4D). In vitro, miRNA-483-5p promoted NSCLC cell migration and invasion, according to these findings.

HIPK2 targets straightforwardly to miRNA-483-5p in NSCLC

To investigate molecular mechanisms underlying miRNA-483-5p advanced tumor cell progression, we searched for putative miRNA-483-5p targets by bioinformatics analysis on TargetScan. We found HIPK2 was a potential applicant focus of miRNA-483-5p in NSCLC. Next, we verified the levels of HIPK2 in NSCLC tissues and cell lines. The NSCLC tissues HIPK2 expression levels were lower than in normal tissues, according to RT-qPCR analysis. (Fig. 5A). The same results were obtained in cell lines (Fig. 5B). By employment of bioinformatics tool TargetScan, we tracked down the putative restricting locales of HIPK2 with miRNA-483-5p (Fig. 5C). Thus, interaction between miRNA-483-5p and HIPK2 were recognized by Luciferase reporter assays. We created a luciferase reporter plasmid containing a miRNA-483-5p potential binding site in the 3'-UTR of HIPK2. The results showed that miRNA-483-5p mimic suppressed the reporter's luciferase operation, whereas the influence of miRNA-483-5p on luciferase expression was lost when the binding site in HIPIK2 3'UTR was mutated (Fig. 5D), indicating that miRNA-483-5p directly attacked the 3'-UTR of HIPK2 in NSCLC. In addition, HIPK2 displayed negative relationship with miRNA-483-5p (Fig. 5E, F). The results revealed that HIPK2 targets directly to miRNA-483-5p in NSCLC.

By inhibiting HIPK2, miRNA-483-5p promotes NSCLC cell proliferation, migration, and invasion while suppressing apoptosis by targeting HIPK2 in vitro

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We generated a pcDNA3.1 HIPK2 vector or si-HIPK2 to investigate a portion of HIPK2's cooperation with miRNA-483-5p in NSCLC cell progression. The RT-PCR assay showed that the miRNA-483-5p expression level was down-regulated significantly in miRNA-483-5p inhibitor group, while there were no differences between inhibitor + si-HIPK2 group ($P < 0.01$). The HIPK2 level was significantly higher in the miRNA-483-5p inhibitor group compared to the inhibitor NC group ($P < 0.01$), but significantly lower in the si-HIPK2 group compared to the si-NC group ($P < 0.01$). However, the decrease was reversed in the miRNA-483-5p inhibitor + si-HIPK2 group ($P < 0.01$). (Fig. 6A). Moreover, miRNA-483-5p expression level was up-regulated in miRNA-483-5p mimics or miRNA-483-5p mimics + pcDNA3.1 HIPK2 compared with miRNA-483-5p mimics NC group ($P < 0.01$); whereas, there were no significant differences between miRNA-483-5p mimics group and mimic + pcDNA3.1 HIPK2 group. The HIPK2 level was significantly lower in miRNA-483-5p mimics compared to miRNA-483-5p mimics NC group ($P < 0.01$), and significantly higher in pcDNA3.1 HIPK2 group compared to pcDNA3.1 group ($P < 0.01$), but the trend was reversed in the miRNA-483-5p mimics + pcDNA3.1 HIPK2 group ($P < 0.01$). (Fig. 6B). These results indicated that miRNA-483-5p was an upstream target of HIPK2 and could negatively regulate HIPK2 expression in A549 cells. The abilities of A549 cells to proliferate, migrate and invade were promoted while apoptosis rate was inhibited in the Si-HIPK2 group, however, the variance could be partially reversed by co-transfection with miRNA-483-5p inhibitor (Fig. 6C-E, Fig. 7). Similarly, the cell proliferation, migration and invasion were enhanced while apoptosis was inhibited in pcDNA3.1 HIPK2 group; whereas, the variance could be partially counteracted by co-transfection with miRNA-483-5p mimic (Fig. 6C-E, Fig. 7). According to these results, miRNA-483-5p promotes NSCLC cell migration, proliferation, and invasion while suppressing apoptosis by inhibiting HIPK2.

Discussion

The formation and progression of tumors have been related to abnormal miRNAs expression levels [17–21]. MiRNA-483-5p express diversely and apply various consequences for an assortment of disease types. Diabetic nephropathy, prostate cancer, lung cancer, and other disorders have been shown to upregulate MiRNA-483-5p [22–24]. MiR-483 family aided tumorigenesis by encouraging cell proliferation, invasion, and resistance to drugs [25, 26]. The mechanisms underlying miRNA-mediated cellular process are attributed to combining to 3'-UTR of the target mRNA [12]. In nasopharyngeal disease, VHL, UBE3A, and UBE3B associated with ubiquitin-mediated proteolysis are target qualities genes of miRNA-483-5p [27]. Despite this, knowledge of the miRNA-483-5p-interceded tumorigenesis in lung cancer is still limited.

According to the results, MiRNA-483-5p was higher in both NSCLC tissues and cell lines. In vitro, knocking out miRNA-483-5p stopped cells from proliferating, migrating, or invading while facilitating apoptosis. To learn more about the effects of miRNA-483-5p on NSCLC tumor progression, we used TargetScan to search for miRNA-483-5p target genes, and discovered that the 3'-UTR of HIPK2 was a promising target site that interacted with miRNA-483-5p. The 3'-UTR of HIPK2 was specifically targeted by miRNA-483-5p, according to a luciferase reporter survey.

HIPK2 acted as a redox signal modulator and participated in scavenger of reactive oxygen species (ROS) [28]. Lately, HIPK2 has been accounted for to be identified with different threatening tumors [14]. HIPK2 was found to reduce ROS development and activate G2/M stage capture, effectively suppressing lung cancer cell migration, proliferation, and invasion [28]. When HIPK2 was combined with miRNA-483-5p, HIPK2 expression was reduced in NSCLC cell lines and was dominated by miRNA-483-5p. According to our results, overexpressing HIPK2 in A549 cells inhibited cell migration, proliferation, invasion, and induced apoptosis. Notwithstanding, miRNA-483-5p HIPK2s reestablished the impact of HIPK2 on this capacity somewhat, which suggested that miRNA-483-5p adversely managed HIPK2 to contributed NSCLC progression.

Conclusions

Taken together, MiRNA-483-5p was effectively up-regulated in NSCLC tissues and cells. MiRNA-483-5p was discovered to promote proliferation, migration, invasion, and stifle apoptosis in NSCLC by controlling HIPK2 expression, according to further research. These results indicated that miRNA-483-5p functions as a tumor enhancer and may be used to treat NSCLC.

Abbreviations

NSCLC: Non-small cell lung cancer; miRNAs: MicroRNAs; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; CCK-8: cell counting kit-8; TUNEL: TdT-mediated dUTP nick end labeling.

Declarations

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Availability of data and material

On fair request, the author will make the datasets used in this study accessible.

Ethics approval and consent to participate

Every persistent gave their permission to participate, and The Wujiang Hospital at Nantong University gave their approval to this project.

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Consent for publication

Not applicable.

Competing interests

The authors announce no irreconcilable interest.

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Figures

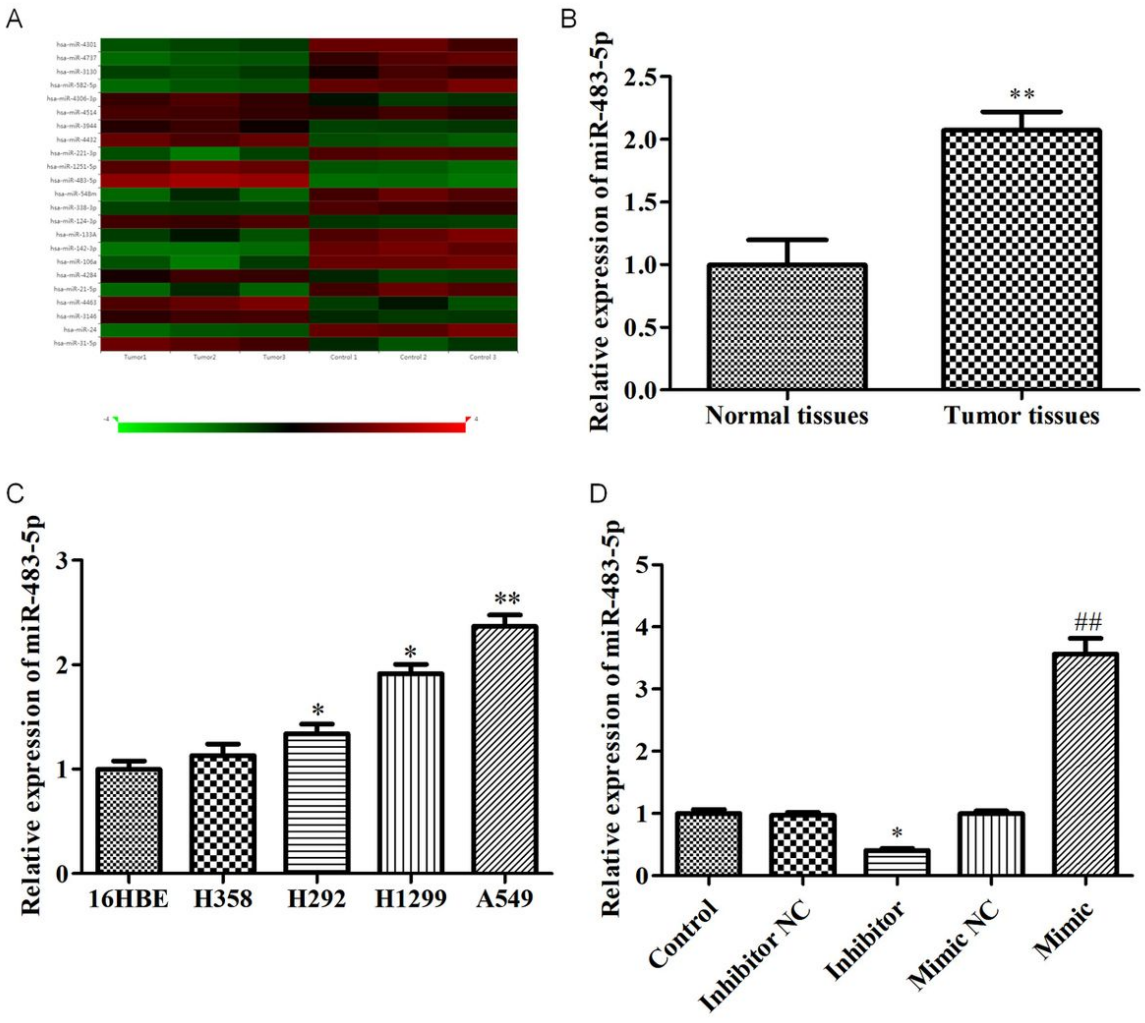

Figure 1

Expression of miRNA-483-5p is higher in NSCLC cell lines and tissues. (A) Heatmap of NSCLC-related microRNA expression profiles. (B-C) RT-qPCR was applied to quantify the NSCLC tissues and cell lines miRNA-483-5p expression. *P < 0.05, **P < 0.01 mean vs the normal tissues or 16HBE cells. (D) The degrees of NSCLC cell lines miRNA-483-5p transfected with miRNA-483-5p inhibitor, mimic or NC. *P < 0.05 mean vs inhibitor NC. #P < 005 mean vs the mimic NC.

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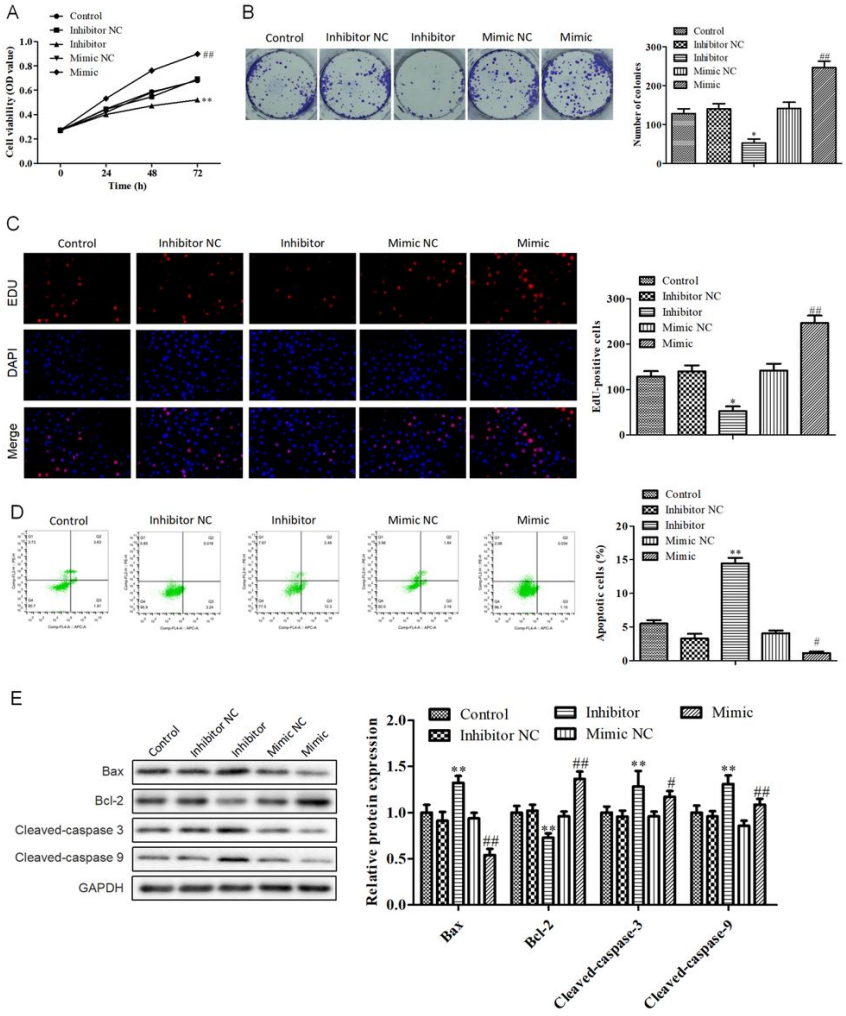

Figure 2

In vitro, miRNA-483-5p promotes NSCLC cell proliferation thus suppressing apoptosis. (A) CCK-8 examine; (B) Colony formation measurement; (C) EdU test; (D) Apoptosis test; (E) Western blotting in A549 cell lines for Bcl-2, Bax, Cleaved-caspase-3/9 protein level transfected with miRNA-483-5p inhibitor, mimic or NC. *P < 0.05, **P < 0.01 mean vs inhibitor NC, #P < 0.05. ##P < 0.01 mean vs mimic NC.

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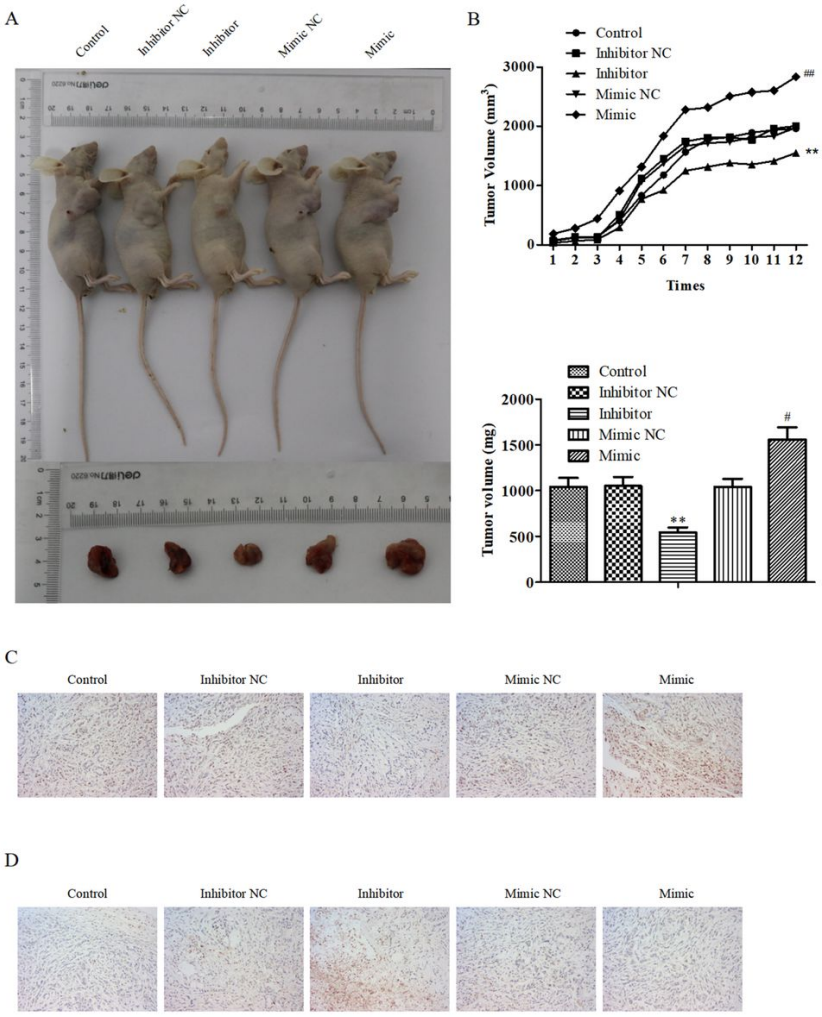

Figure 3

Up-regulated of microRNA-483-5p promoted tumor growth in vivo. (A) The mice were pictured. (B) At 5th weeks after infusion, tumors were removed, measured and visualized. (C) Immunohistochemistry determined the expression level of Ki67. (D) Tunel assay determined the apoptosis level. **P < 0.01 mean vs inhibitor NC inhibitor, #P < 0.05. ##P < 0.01 mean vs mimic NC.

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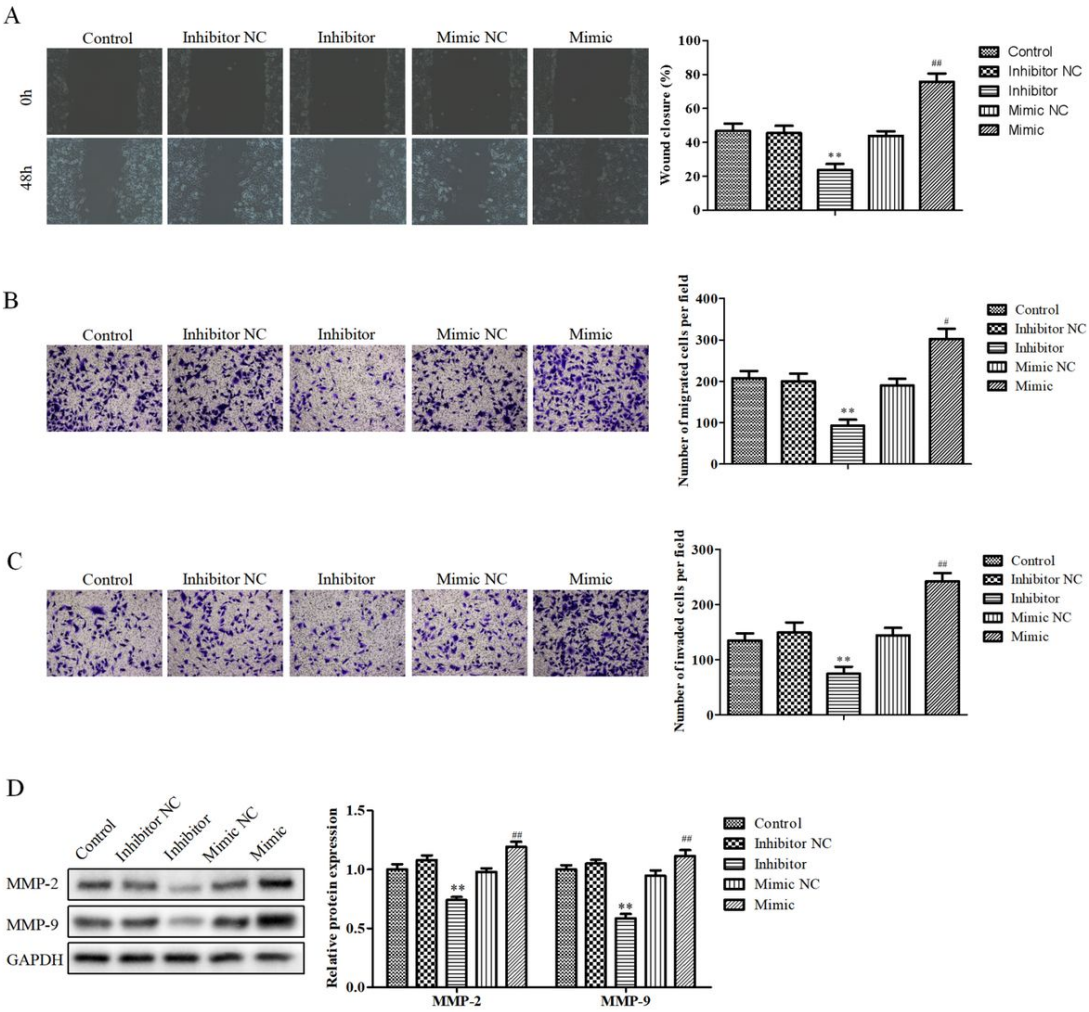

Figure 4

NSCLC cell proliferation, migration and invasion are aided by miRNA-483-5p in vitro. (A) Scratch test; (B, C) Transwell test; (D) Western blotting in A549 cells for MMP-2 and MMP-9 protein expression level transfected with miRNA-483-5p inhibitor, mimic or NC. **P < 0.01 mean versus inhibitor NC, #P < 0.05. ##P < 0.01 mean vs. mimic NC.

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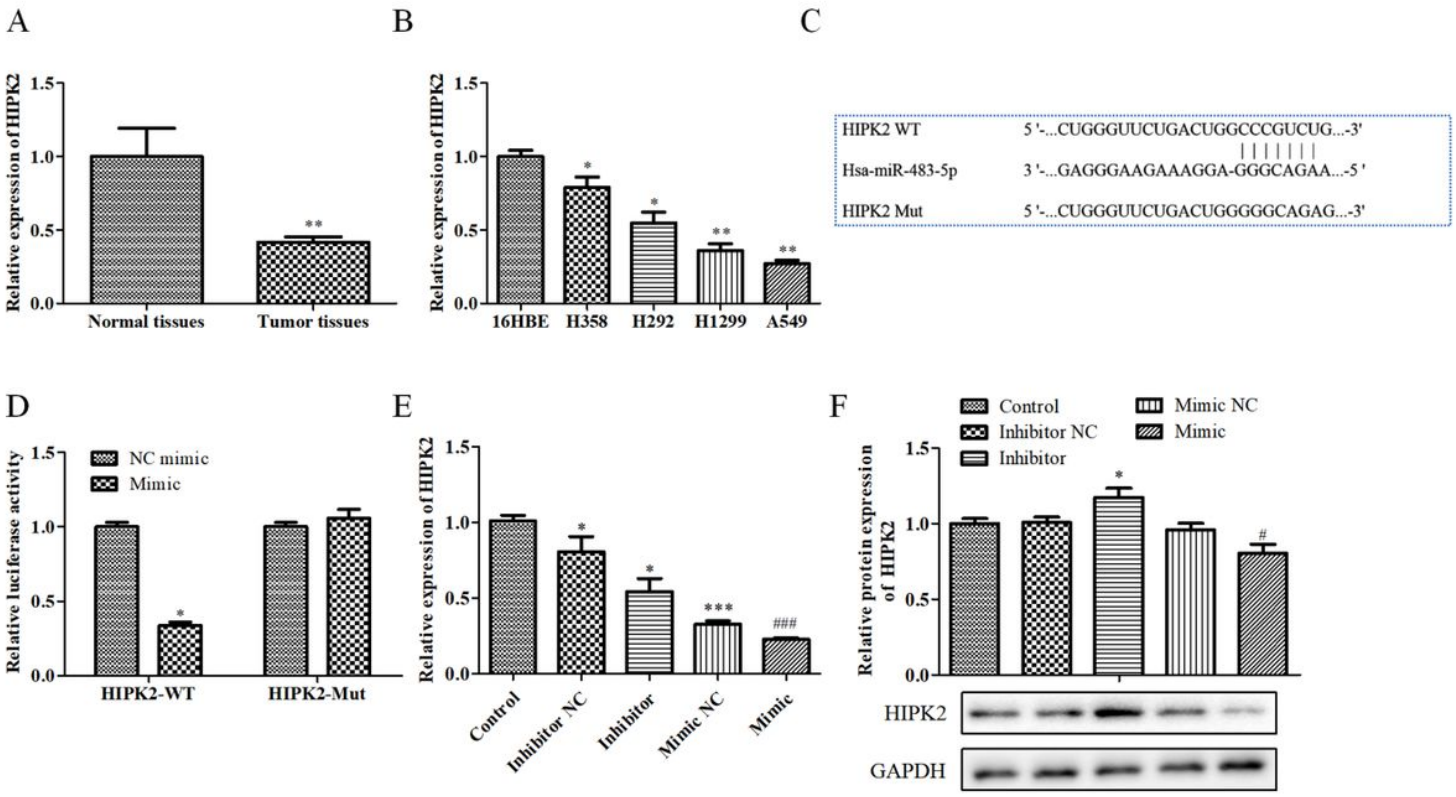

Figure 5

MiRNA-483-5p straightforwardly targets HIPK2 in NSCLC. (A, B) In NSCLC tissues and cell lines, the HIPK2 expression was tested. *P < 0.05, **P < 0.01 versus normal tissues or 16HBE cells. (C) Predicted restricting locales in the 3'-UTR of HIPK2 mRNA and seed succession of miRNA-483-5p. (D) Dual luciferase report assay. *P < 0.05, mimic vs. NC mimic. (E, F) In cell lines, HIPK2 expression is negatively regulated by miRNA-483-5p. *P < 0.05 mean vs. inhibitor NC, #P < 0.05. ##P < 0.01 mean vs. mimics NC.

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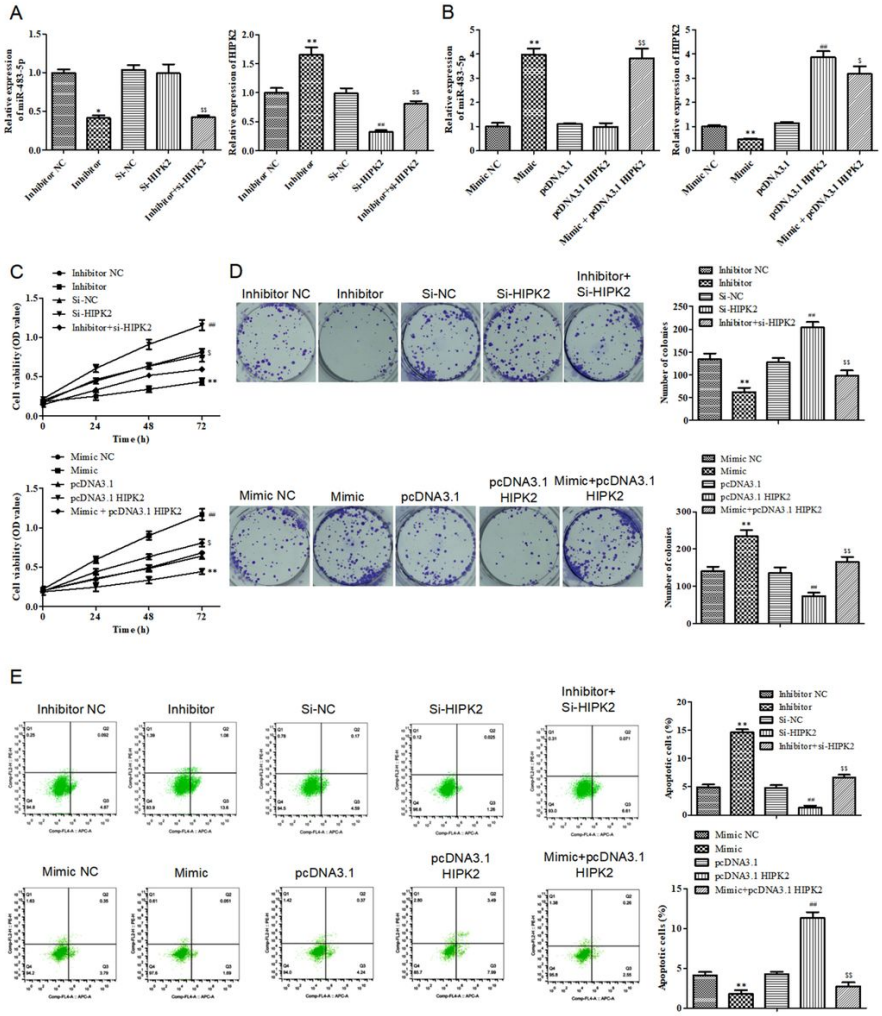

Figure 6

In vitro, miRNA-483-5p inhibits HIPK2 and encourages NSCLC cell proliferation while suppressing apoptosis. (A, B) The miRNA-483-5p and HIPK2 expressions in A549 cells after transfected with NC, miRNA-483-5p inhibitor, si-HIPK2, miRNA-483-5p mimic, pcDNA3.1-HIPK2. *P < 0.05, **P < 0.01, mean vs. Inhibitor NC; ##P < 0.01 mean vs. Si-NC; $P < 0.05,

P < 0.01 mean vs. pcDNA3.1 HIPK2. (C) CCK-8 test; (D) colony formation test; (E) Apoptosis test. *P < 0.05, **P < 0.01, mean vs. Inhibitor NC or mimic NC; #

P < 0.01 mean vs. si-HIPK2 or pcDNA3.1-HIPK2.

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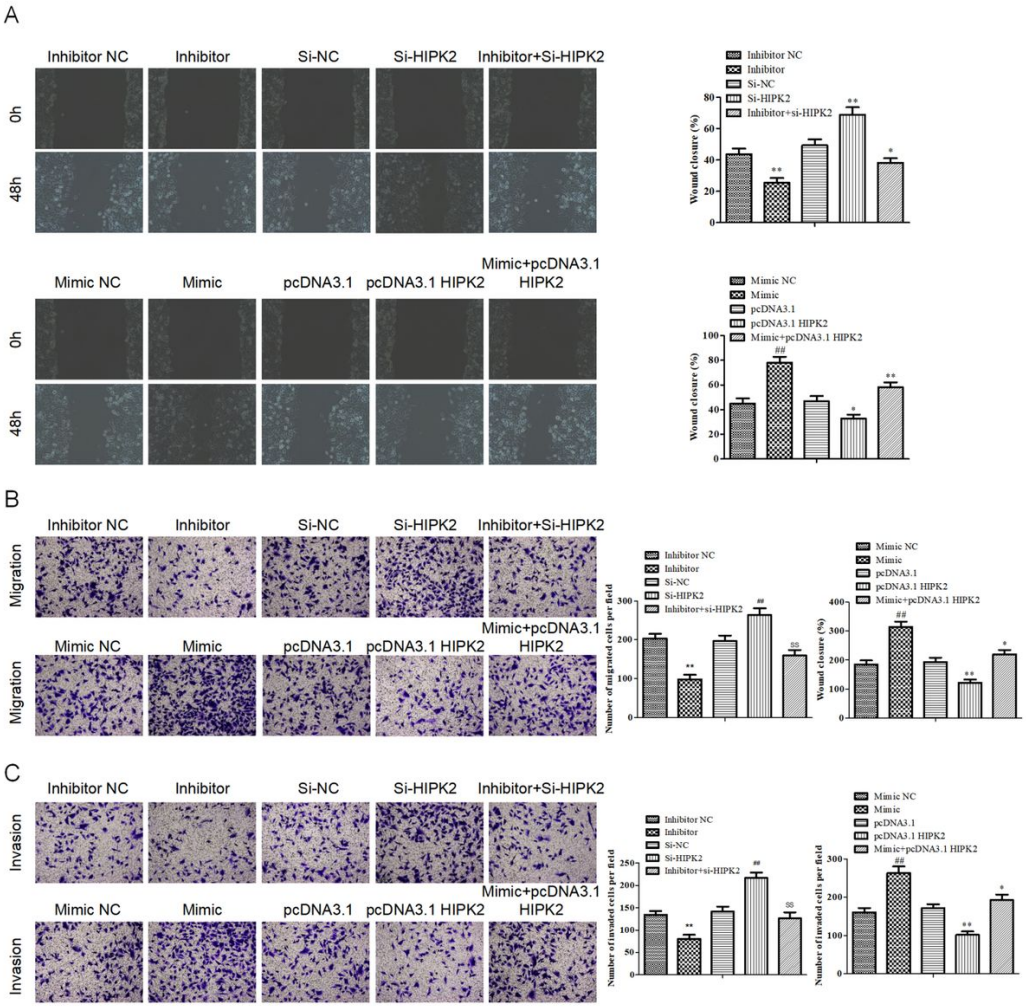

Figure 7

MiRNA-483-5p suppresses apoptosis while promotes proliferation, cell migration, and invasion in NSCLC cells by inhibiting HIPK2. (A) Scratch assay; (B, C) Transwell invasion assay in A549 cell lines transfected with inhibitor NC, miRNA-483-5p inhibitor, Si-NC, Si-HIPK2, pcDNA3.1 and pcDNA3.1-HIPK2. **P < 0.01 vs. Inhibitor NC or Mimic NC; #P < 0.05, ##P < 0.01 mean vs. Si-NC or pcDNA3.1; $P < 0.05, $$P < 0.01 mean v. si-HIPK2 or pcDNA3.1-HIPK2.

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