# Long non-coding RNA HULC regulates growth and metastasis of human glioma cells via induction of apoptosis and inhibits cell migration and invasion

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## Abstract

**Introduction:** The long non-coding RNA HULC has been shown to be involved in the development of several human cancers. The present study was undertaken to investigate the regulatory role of lncRNA-HULC in growth and metastasis of human glioma.
**Material and methods:** The gene expression of lncRNA-HULC was estimated from the clinical glioma tissues and cell lines using RT-PCR. The proliferation of transfected cancer cells was determined with the help of cell counting kit-8 (CCK8). DAPI staining and dual annexin V-FITC/PI staining procedures were used for inferring the apoptosis of transfected cancer cells. Scratch-heal and transwell chamber assays were employed for the determination of migration and invasion of transfected cells. The expression of proteins of interest was studied by western blotting technique.
**Results:** The results showed that lncRNA-HULC exhibits significantly ($p < 0.05$) higher expression in glioma tissues and cancer cells. The knockdown of lncRNA-HULC led to a marked decline in the proliferation of glioma cells through apoptotic induction which was accompanied by upregulation of Bax and downregulation of Bcl-2. Moreover, knockdown of lncRNA-HULC significantly ($p < 0.05$) suppressed the migration and invasion of cancer cells *in vitro*. The western blot analysis showed that lncRNA-HULC exerted its effects via modulation of the PI3K/AKT signaling pathway.
**Conclusions:** The study revealed the possibility of targeting the PI3K/AKT signaling pathway in glioma through transcriptional knockdown of lncRNA-HULC, which might be utilized for therapeutic purposes against human glioma.

**Key words:** glioma, long non-coding RNA, proliferation, apoptosis, transcriptional knockdown, migration, invasion.

## Introduction

The gliomas include the most dominant form of endocrine tumors affecting the human brain and cause tremendous human mortality at the global level [1]. Histologically, the human gliomas are deemed to emerge from the neuro-epithelial tissue and are categorized based on clinical features and histo-pathological parameters [2]. Among the sub-types of glioma, the glioblastoma is considered to be the most lethal and an extremely aggressive tumor [3]. The average survival period of glioblastoma patients is less than 1 year after disease diagnosis [4]. Currently,

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patients with glioma are treated with the combinatorial application of surgical and chemo and/or radiotherapeutic procedures. This combined therapeutic approach has enhanced the survival rate of glioma, but satisfactory clinical outcomes are yet to be achieved. Therefore, it is crucial to understand the in-depth pathology of glioma at the molecular level to develop more efficient therapeutic approaches against this lethal malignancy. During the recent era, research findings have broadened our understanding and knowledge about the cancer and have led to the identification of vital regulatory players responsible for controlling the growth and progression of human cancers. Such studies also showed the potential of the long non-coding RNAs (lncRNAs) to serve in the prognosis of various cancerous disorders together with their utility as potent anti-cancer therapeutic biomolecules [5]. LncRNAs are described as RNA molecules, ranging in size from 200 to more than 100 thousand nucleotides in length, which do not code for proteins but function as transcriptional regulators [6]. It has been reported that lncRNAs assist in tissue homeostasis and regulate the human cancer progression besides serving other biological and physiological roles [7]. A good number of studies have shown that aberrant transcript levels of lncRNAs profoundly affect the initiation and progression of human gliomas [8, 9]. In this regard, we tried to investigate the regulatory effects of lncRNA-HULC in controlling the growth and spread of glioma by taking advantage of the cell line system. The lncRNA highly up-regulated in liver cancer (HULC) was identified as the lncRNA most up-regulated expressed in hepato-cellular carcinoma in 2007 [10]. It is located at the chromosomal section 6p24.3 and has a length of about 500 nucleotides consisting of two exons. LncRNA-HULC has been suggested to play a critical role in various human cancers. It is shown to modulate the HBx/STAT3/miR-539/APOBEC3B signaling pathway to regulate HBV-related hepatocellular carcinoma [11]. LncRNA-HULC down-regulates miR-15a to promote growth, migration and invasion of pancreatic cancer cells and acts as a prognostic factor of pancreatic cancer [12, 13]. However, its role in glioma is still not well known. Thus, the present study was aimed at the exploration of this together with determination of the molecular mechanism mediating the regulatory role of lncRNA-HULC in human glioma. The results revealed lncRNA-HULC as an important regulator of growth and metastasis of human glioma.

**Table I.** List of primers used in the study

| Primer | Direction | Sequence |
|---|---|---|
| LncRNA | Forward | 5′-TCATGATGGAGCCTT-3′ |
| | Reverse | 5′-CTCTTCCTGGCTTGCAGATTG-3′ |
| β-actin | Forward | 5′-CCTGGATAGC AACGTAC-3′ |
| | Reverse | 5′-CACCTTCTACAATGAGCT-3′ |

## Material and methods

### Procurement of clinical tissues and culturing and transfection of cell lines

The normal brain tissues adjacent to gliomas and glioma tissues were taken from the patients after their surgical excision at the Department of Neurosurgery, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai, China. The patients were informed in advance and procurement of clinical specimens was made only after written consent had been signed by them. Standard scientific ethical guidelines were followed strictly for the experimental usage of these tissues. The research ethics approval number for experimentation on human tissues was FUF/0881/2019. Liquid nitrogen was used for the transportation of specimens and their storage was done in ultra-low temperature freezers. All the human glioma cell lines (U87, MO59K, U118, Hs683 and LN18) along with the normal astrocyte cell line were obtained from the Cell Bank of the Chinese Academy of Science. The culturing of all the cell lines was performed using high glucose supplemented DMEM growth medium (Thermo Fisher Scientific). A humidified incubator was used for growing the cell lines at 37°C. Lipofectamine 2000 (Thermo Fisher Scientific) reagent was used to perform the transfection of cell lines with specific transfection constructs. The lncRNA-HULC silencing construct (si-HULC) as well as the normal control silencing construct were purchased from the RiboBio biotechnology company.

### RNA isolation and RT-PCR study

Using TRIzol reagent, the total RNA was isolated from the clinical tissues and cell lines. After its treatment with DNase I and quantity estimation, 2.5 µg RNA was used to synthesize the cDNA with the help of a high capacity cDNA synthesis kit (Thermo Fisher Scientific). Then the expression of lncRNA-HULC was analyzed using the One Step SYBR Prime Script PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) through the quantitative real time-PCR method. The relative expression levels were ascertained by $2^{-ddCt}$ method based calculations. The human β-actin gene was used as an internal control in the expression analysis. The primers used are listed in Table I.

### Proliferation assay

The transfected glioma cells were added to 96-well plates at the density of $2 \times 10^3$ cells/well. The

cells were cultured for either 0 h, 12 h, 24 h, 36 h, 48 h, 72 h or 96 h at 37°C. Cell proliferation rate was determined with the help of Cell Counting Kit-8 (CCK-8, Sigma-Aldrich). The absorbance was measured for each sample at 450 nm. The OD values were then used to draw the proliferation rate curves for their relative comparison.

### Analysis of cell apoptosis

The transfected glioma cells were cultured for 48 h in 12-well plates at a density of $3 \times 10^5$ cells/well. Next, the cells were collected by centrifugation, washed with cold PBS, stained with 4′,6-diamidino-2-phenylindole (DAPI) fluorescent staining solution and visualized under the fluorescent microscope for nuclear fluorescence to infer the level of apoptosis. The apoptosis levels were also analyzed through flow cytometry. Here the cells were stained with dual annexin V-FITC/PI staining mix prior to their flow cytometric investigation using the FITC Annexin V apoptosis detection kit (BD Bioscience).

### Cell migration and invasion assays

For the analysis of migration of glioma cells transfected with specific transfection constructs, the cells were grown in 6-well plates at 37°C until the regular cell surface was obtained. Using the 200 µl pipette tip, the cell surface was perpendicularly scratched and photographed through a light microscope. The plate was incubated at 37°C for 24 h after which the scratch line was again observed under the microscope, photographed and compared with the initial scratch width. The invasion of transfected glioma cancer cells was determined using the transwell chamber plate fitted with Matrigel. After obtaining the homogeneous suspension by trypsinization, 250 µl of the suspension of transfected cells, with cellular density of $2.5 \times 10^6$ cells/ml, was added to the upper part of the transwell chamber. The lower part of the chamber was given 750 µl of growth medium only. The plate was incubated at 37°C for 48 h, and after the incubation, the cells invading the lower surface of the membrane were fixed with 70% ethanol, and then stained with Giemsa solution. The stained cells were visualized using an inverted microscope, photographed and the percentage of invasion was calculated using five random microscopic fields.

### Protein expression study

The cell lysates were obtained by treating the transfected cells with RIPA lysis buffer. These extracts were separated on 10% SDS–PAGE gel which was blotted to PVDF membranes. Skimmed milk was used for blocking the membranes. Membranes were given the exposure of primary antibodies (Bax Cat#2774S, Bcl-2 Cat#42234S, PI3K Cat#4292, p-PI3K Cat#4228S, Akt Cat#9272, p-AKT Cat#4060 and β-actin Cat#4967, dilutions of 1 : 1000, (Cell Signaling Technology, CST, USA)) in dark at 4°C for 12 h, following which they were incubated with respective horseradish peroxidase conjugated secondary antibodies. With the help of enhanced chemiluminescence substrate, the protein bands were detected to infer their concentrations. Human β-actin protein was used to serve as the internal control in the protein expression studies.

### Statistical analysis

The mean and standard deviation were calculated from the values obtained for a specific parameter and the final value was presented as mean ± standard deviation. The statistical analysis was performed using GraphPad prism 7.0 software. Student's *t* test was performed and *p* at $< 0.05$ was taken to represent a statistically significant level of difference between two values.

## Results

### lncRNA-HULC is highly expressed in glioma

The qRT-PCR analysis was used to determine the expression of lncRNA-HULC in the clinical tissues – normal and glioma. The results showed that glioma tissues exhibited significantly higher expression of lncRNA-HULC (Figure 1 A). Similarly, the study of expression of lncRNA-HULC from the glioma cell lines (U87, MO59K, U118, Hs683 and LN18) in comparison to normal astrocytes hinted towards the same inference, i.e., all the glioma cell lines showed significantly higher lncRNA-HULC expression than the normal astrocytes (Figure 1 B). The cancer cell lines U87, MO59K, U118, Hs683 and LN18 respectively exhibited 3.7, 4.5, 4.3, 3.2 and 3.4 fold higher transcript levels of lncRNA-HULC than normal astrocytes. Together, the results suggested the probable regulatory role of lncRNA-HULC in human glioma.

### lncRNA-HULC knockdown reduced proliferation of glioma cells through apoptotic induction

To evaluate the effect of lncRNA-HULC on proliferation of the glioma cells, knockdown of lncRNA-HULC through transfection was carried out in two glioma cell lines, MO59K and U118. The relative expression of lncRNA-HULC was only 0.2 and 0.18 in MO59K and U118 cell lines, respectively, in comparison to the negative control cells (transfected with si-NC). The silencing of lncRNA-HULC was confirmed by the RT-PCR anal-

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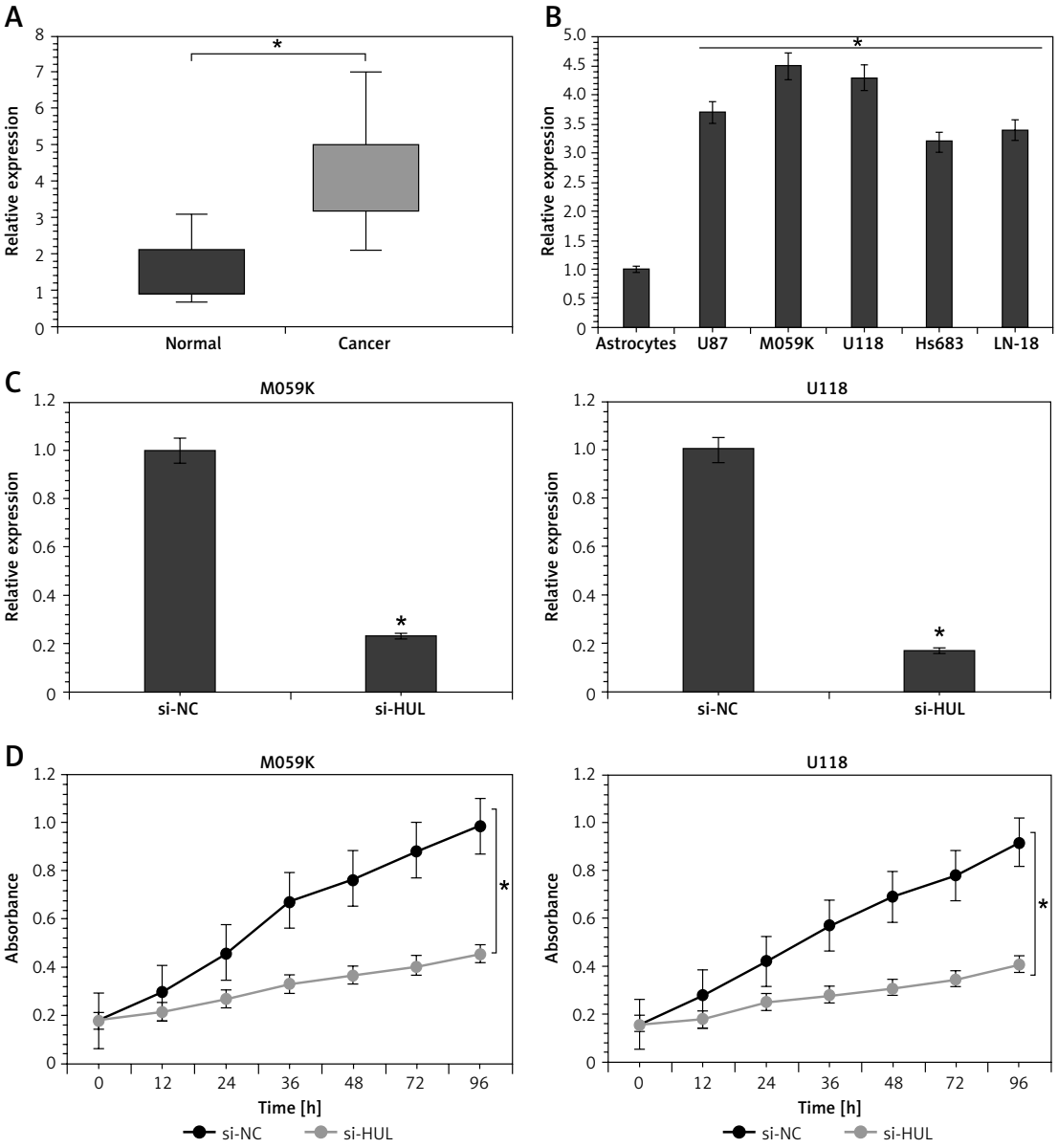


**Figure 1.** LncRNA-HULC is highly expressed in human glioma and its transcriptional knockdown markedly reduces the proliferation of glioma cells *in vitro*. **A** – Expression analysis of lncRNA-HULC in normal and glioma tissues. **B** – Expression analysis of lncRNA-HULC in normal astrocytes and glioma cancer cell lines (U87, MO59K, U118, Hs683 and LN18). **C** – Expression analysis of lncRNA-HULC in MO59K and U118 cancer cells transfected with si-NC or si-HULC. **D** – Cell counting kit-8 based assessment of proliferation of MO59K and U118 cancer cells transfected with si-NC or si-HULC. The experiments were performed in triplicate and expressed as mean ± SD (*$p < 0.05$)

ysis (Figure 1 C). The cell counting kit-8 based estimation of proliferation assessment of cancer cells under lncRNA-HULC knockdown in comparison to the respective normal control cells showed that silencing of lncRNA-HULC actively decreased the proliferation of glioma cancer cells and the effects were more prominent when higher growth incubation periods were used (Figure 1 D). Now to look for the underlying basis of proliferation decline, a study of cell apoptosis was performed using DAPI staining. It was noted that the glioma cancer cells, MO59K and U118 showed clear signs of nuclear lesions under lncRNA-HULC knockdown indicating the induction of apoptosis (Figure 2 A). The induction of apoptosis in cancer cells under lncRNA-HULC gene silencing was also inferred from their flowmetric investigation. The percentage of apoptotic cells was significantly higher for the glioma cells under lncRNA-HULC knockdown in comparison to the normal control cells (Figure 2 B). The final support was gained from the blotting study of Bax and Bcl-2 apoptosis related proteins where Bax protein expression was seen to be enhancing under the knockdown of lncRNA-HULC while Bcl-2 was shown to be decreasing (Figure 2 C). The results thus clearly reveal that lncRNA-HULC gene

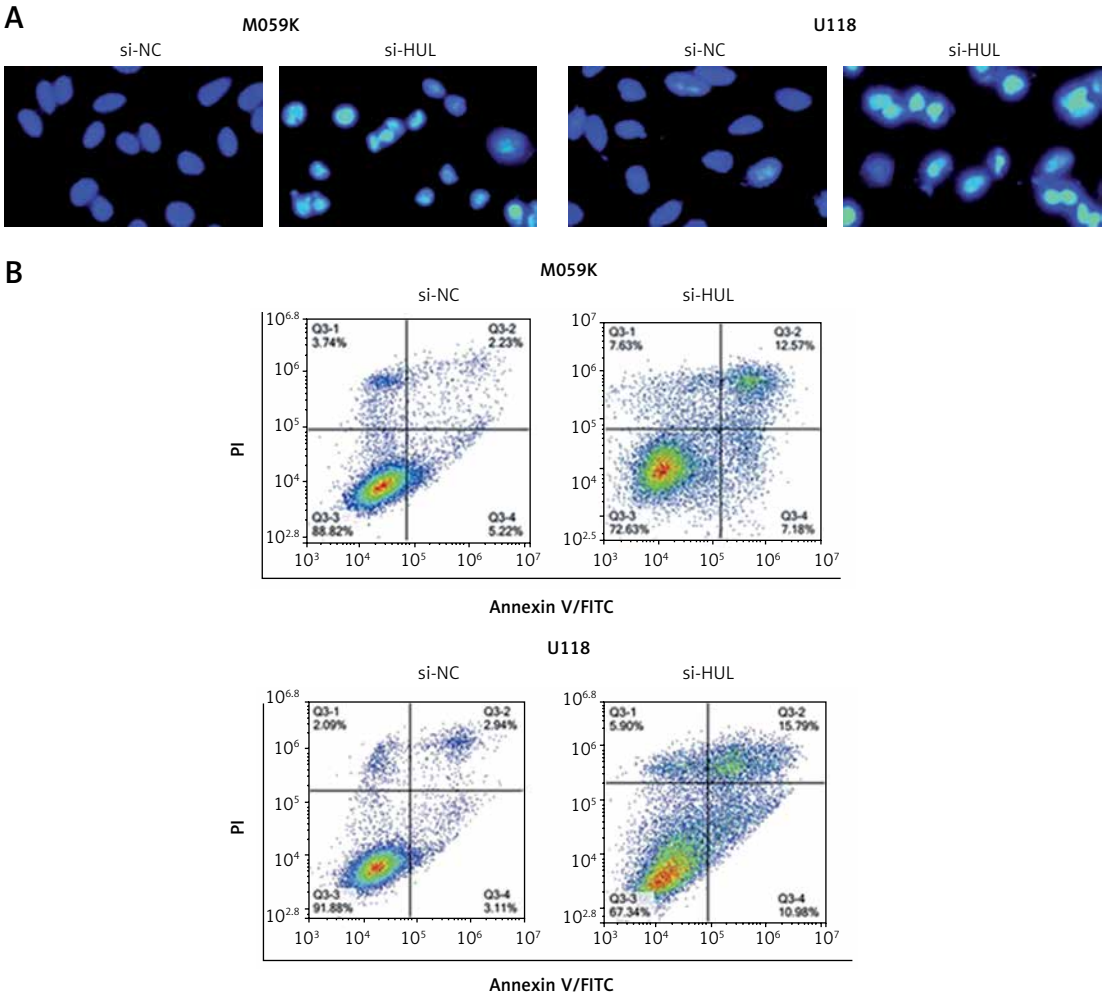


**Figure 2.** LncRNA-HULC knockdown induces apoptosis in glioma cancer cells. **A** – DAPI staining for morphological assessment of nuclei of MO59K and U118 cancer cells transfected with si-NC or si-HULC. **B** – Flow cytometric analysis for apoptosis of annexin V-FITC/PI dual stained MO59K and U118 glioma cancer cells transfected with si-NC or si-HULC. **C** – Western blotting of Bax and Bcl-2 proteins from MO59K and U118 cancer cells transfected with si-NC or si-HULC. The experiments were performed in triplicate

silencing in glioma cells has a potential to reduce the growth of glioma cells by significant levels through apoptotic induction.

## Silencing of lncRNA-HULC inhibited migration and invasion of glioma cancer cells

The effects of lncRNA-HULC knockdown were also studied on the glioma cancer cell motility. It was found that the silencing of lncRNA-HULC significantly reduced the migration of the glioma cancer cells MO59K and U188 (Figure 3). The invasion of the glioma cancer cells was also observed to fall markedly when knockdown of lncRNA-HULC was carried out (Figure 4). Both the results depict that lncRNA-HULC might be controlling the metastasis of human glioma and its silencing might thus actively be able to check the disease spread.

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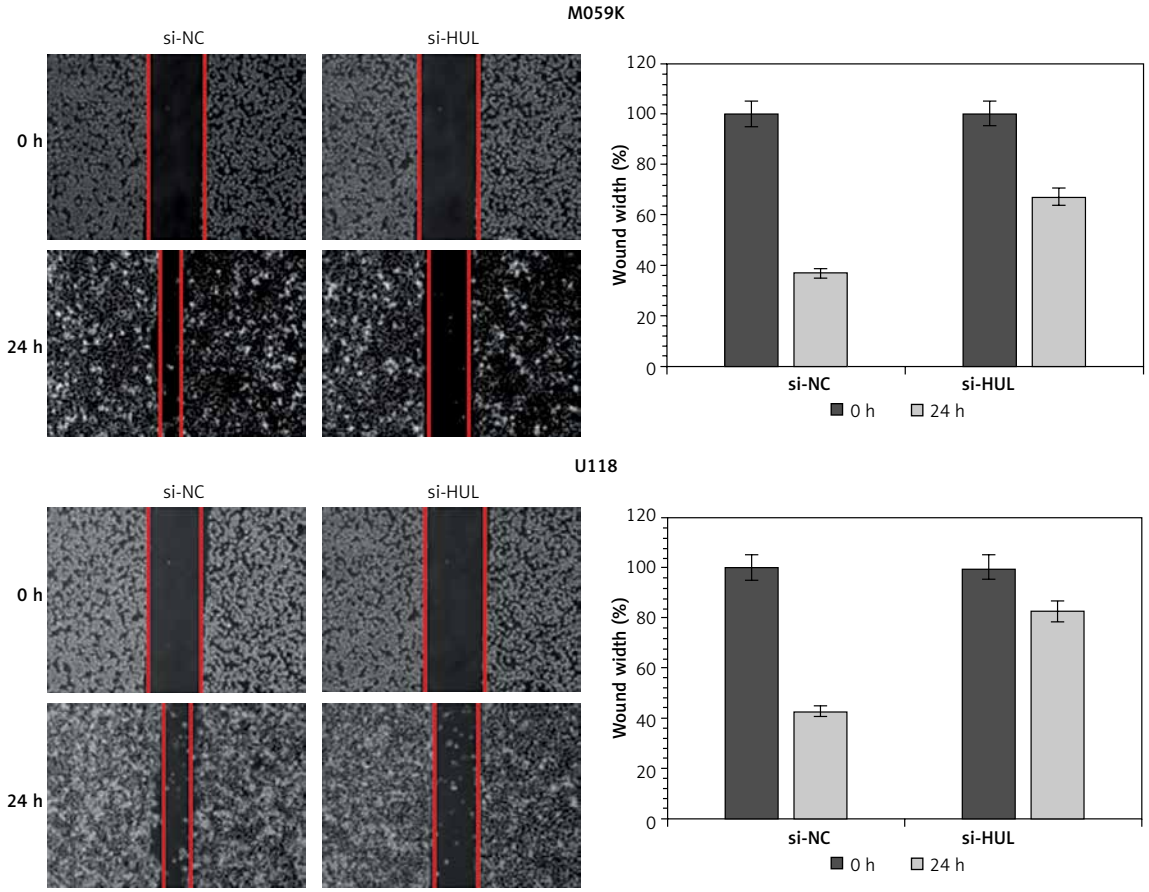


**Figure 3.** Silencing of lncRNA-HULC restricts the migration of glioma cancer cells. Scratch-heal assay for the estimation of migration of MO59K and U118 cancer cells transfected with si-NC or si-HULC. The experiments were performed in triplicate and expressed as mean ± SD (*$p < 0.05$)

### The PI3K/AKT signaling pathway was blocked under lncRNA-HULC gene silencing

To try to determine the underlying mechanism through which lncRNA-HULC might be operating to regulate glioma cancer cell growth, western blotting of crucial components of the PI3K/AKT signaling pathway was performed. The results showed that when knockdown of lncRNA-HULC was made, the level of phosphorylated PI3K and AKT proteins significantly declined. However, little or no effect on the protein levels of non-phosphorylated forms of these proteins was seen (Figure 5). The finding thus suggests that lncRNA-HULC might be positively controlling the phosphorylation of PI3K and AKT proteins and its silencing might thus be used to partially block the PI3K/AKT pathway in glioma cells through inhibition of phosphorylation, which also highlights the therapeutic potential of lncRNA-HULC against human glioma growth and progression.

## Discussion

During recent times the application of multiple anti-cancer procedures of surgical resection in combination with the current chemo- and radiotherapies has not only enhanced the survival rate of glioma but has also considerably reduced the suffering of glioma patients. However, satisfactory treatment results have not yet been achieved against this deadly disorder. As such, researchers are continuously in search of better therapeutic procedures, and in this regard, the exploration of various regulatory aspects controlling the growth and spread of human glioma is imperative. The current study was designed in the same direction and our results showed that aberrant expression of lncRNA-HULC might be one of the molecular errors governing the initiation and progression of glioma in humans, as has been proposed for this class of regulatory RNAs in human tumors [14–16]. Our study confirmed the over-expression of lncRNA-HULC in glioma, as was reported by previous research investigations. The knockdown of lncRNA-HULC reduced the proliferation of glioma cancer cells, which was confirmed to result from induction of apoptosis in glioma cells. The results are in line with the previous cancer cell growth reduction reports about lncRNA-HULC and support the proposition of therapeutic application of this

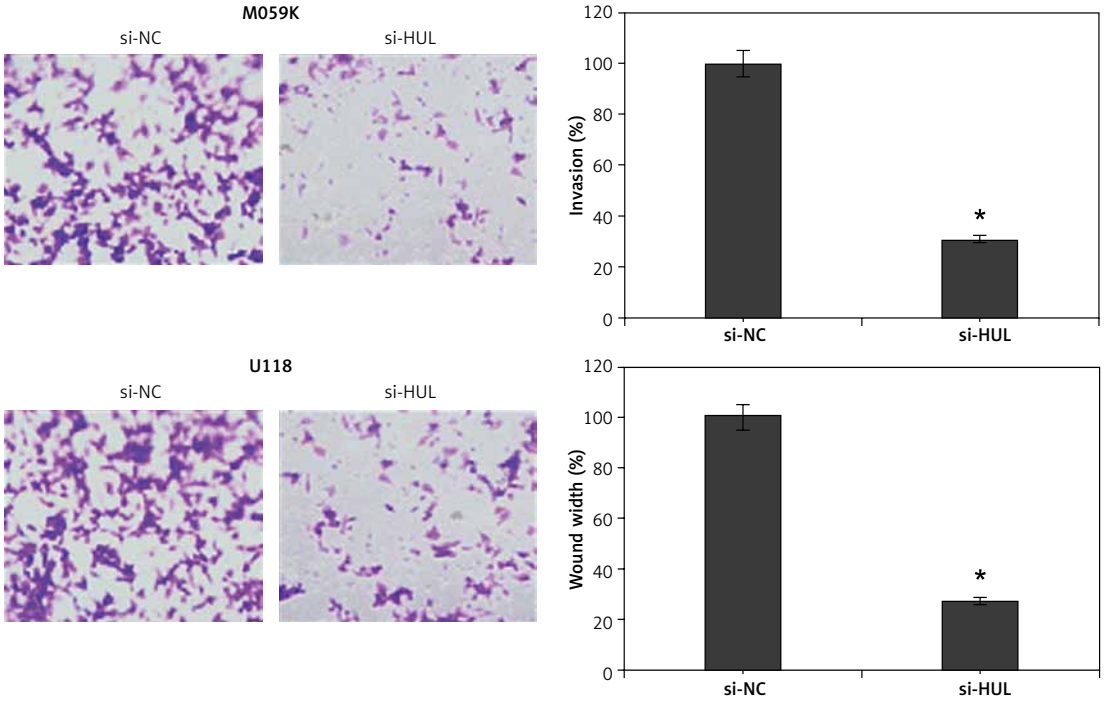


**Figure 4.** LncRNA-HULC knockdown reduces the *in vitro* invasion of glioma cancer cells. Transwell chamber assay for determination of invasion of MO59K and U118 cancer cells transfected with si-NC or si-HULC. The experiments were performed in triplicate and expressed as mean ± SD (*$p < 0.05$)

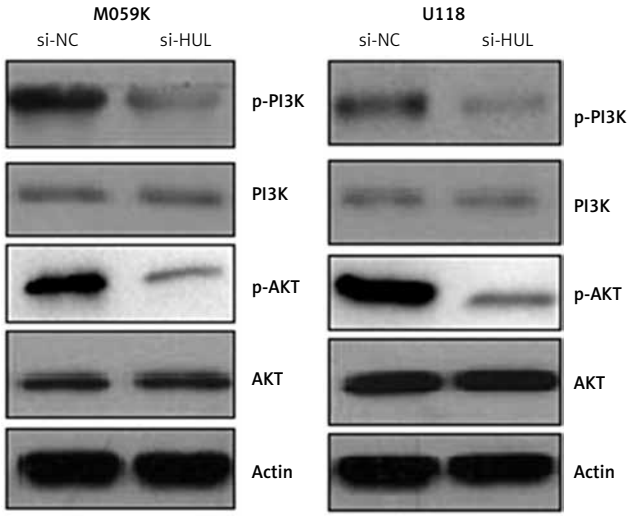


**Figure 5.** PI3K/AKT signaling pathway is inhibited in glioma cancer cells under lncRNA-HULC gene silencing. Western blotting of phosphorylated PI3 and AKT (p-AKT and p-AKT) proteins and their respective non-phosphorylated forms from the MO59K and U118 cancer cells transfected with si-NC or si-HULC. The experiments were performed in triplicate

RNA biomolecule against the human cancer [17, 18]. The treatment of human cancers including gliomas is hindered by some undesirable effects and majorly by the invasion of cancer cells to the surrounding tissues by the process of metastasis. Thus, it is crucial to evaluate measures which might restrict the cancer progression through metastasis. Here, in an interesting finding when the silencing of lncRNA-HULC was performed in the glioma cells, the cancer cells exhibited significantly lower motilities when investigated though *in vitro* assays. Such promising anti-cancer therapeutic results about the role of lncRNA-HULC in human cancers have also been noted by previous researchers [19–21]. Moreover, lncRNA-HULC through its interaction with miR-613 was shown to regulate the growth and metastasis of colon cancer cells in a similar fashion [22]. The sponging of miR-6754-5p by lncRNA-HULC was also reported to promote breast cancer development [23]. The cancer cells show higher growth potential because of the constitutive over-activation of signaling pathways governing the rate of division [24]. The PI3K/AKT signaling pathway is also among such pathways and thus workers have proposed the therapeutic targeting of this crucial pathway as a promising anti-cancer strategy [25]. In the present study, the results highlighted that the PI3K/AKT pathway

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could be indirectly targeted and its signaling intensity might be lowered through transcriptional silencing of lncRNA-HULC. Regulation of the PI3K/AKT signaling pathway in glioma cells has already been confirmed by scientific researchers [26]. On the whole, the results of the current study support the regulatory role of lncRNA-HULC in human glioma together with the possibility of its usage as a vital prognostic and therapeutic molecule against the growth and progression of this aggressive disorder. Although the present study explored the therapeutic implications of LncRNA-HULC in glioma cells, the main limitation of the study is the lack of *in vivo* investigation. Therefore, the results of the present investigation require *in vivo* validation. Furthermore, identification of chemotherapeutic agents which can suppresses the expression of LncRNA-HULC could open new avenues for the treatment of human glioma and remains an important area of investigation.

In conclusion, the findings of the present study are suggestive of significant up-regulation of lncRNA-HULC in human glioma. Interestingly, the results showed that experimental knockdown of lncRNA-HULC induced apoptosis in glioma cells and inhibited their growth and viability. LncRNA-HULC transcriptional silencing also remarkably hampered the migration and invasion of glioma cells. The regulatory effects of lncRNA-HULC were shown to be exerted through the PI3K/Akt signaling pathway. The findings point towards the therapeutic implications of lncRNA-HULC in glioma.

## Conflict of interest

The authors declare no conflict of interest.

## References


1. Lapointe S, Perry A, Butowski NA. Primary brain tumours in adults. Lancet 2018; 392: 432-46.
2. Skjulsvik AJ. On the prognostic factors of low-grade gliomas. NTNU Open 2020.
3. Tivnan A, Heilinger T, Lavelle EC, Prehn JH. Advances in immunotherapy for the treatment of glioblastoma. J Neurooncol 2017; 131: 1-9.
4. You Y, Ru X, Lei W, et al. Developing the novel bioinformatics algorithms to systematically investigate the connections among survival time, key genes and proteins for Glioblastoma multiforme. BMC Bioinformatics 2020; 21: 1-4.
5. Zhang R, Xia LQ, Lu WW, Zhang J, Zhu JS. LncRNAs and cancer. Oncol Letters 2016; 12: 1233-9.
6. Hombach S, Kretz M. Non-coding RNAs: classification, biology and functioning. InNon-coding RNAs in colorectal cancer. Springer, Cham 2016; 3-17.
7. Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell 2016; 29: 452-63.
8. Wu Y, Qian Z. Long non-coding RNAs (lncRNAs) and microRNAs regulatory pathways in the tumorigenesis and pathogenesis of glioma. Discov Med 2019; 28: 129-38.
9. Pastori C, Wahlestedt C. Involvement of long noncoding RNAs in diseases affecting the central nervous system. RNA Biology 2012; 9: 860-70.
10. Panzitt K, Tschernatsch MM, Guelly C, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 2007; 132: 330-42.
11. Liu Y, Feng J, Sun M, et al. Long non-coding RNA HULC activates HBV by modulating HBx/STAT3/miR-539/APOBEC3B signaling in HBV-related hepatocellular carcinoma. Cancer Letters 2019; 454: 158-70.
12. Feng H, Wei B, Zhang Y. Long non-coding RNA HULC promotes proliferation, migration and invasion of pancreatic cancer cells by down-regulating microRNA-15a. Int J Biol Macromolecules 2019; 126: 891-8.
13. Ou ZL, Luo Z, Lu YB. Long non-coding RNA HULC as a diagnostic and prognostic marker of pancreatic cancer. World J Gastroenterol 2019; 25: 6728-42.
14. Park JY, Lee JE, Park JB, Yoo H, Lee SH, Kim JH. Roles of long non-coding RNAs on tumorigenesis and glioma development. Brain Tumor Res Treat 2014; 2: 1-6.
15. Zhang X, Sun S, Pu JK, et al. Long non-coding RNA expression profiles predict clinical phenotypes in glioma. Neurobiol Dis 2012; 48: 1-8.
16. Zhang XQ, Leung GK. Long non-coding RNAs in glioma: functional roles and clinical perspectives. Neurochem Int 2014; 77: 78-85.
17. Peng W, Gao W, Feng J. Long noncoding RNA HULC is a novel biomarker of poor prognosis in patients with pancreatic cancer. Med Oncol 2014; 31: 346.
18. Lu Z, Xiao Z, Liu F, et al. Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). Oncotarget 2016; 7: 241-54.
19. Yu X, Zheng H, Chan MT, Wu WK. HULC: an oncogenic long non-coding RNA in human cancer. J Cell Mol Med 2017; 21: 410-7.
20. Wang Y, Chen F, Zhao M, et al. The long noncoding RNA HULC promotes liver cancer by increasing the expression of the HMGA2 oncogene via sequestration of the microRNA-186. J Biol Chem 2017; 292: 15395-407.
21. Xie H, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. BioMed Res Int 2013; 2013: 136106.
22. Dong Y, Wei MH, Lu JG, Bi CY. Long non-coding RNA HULC interacts with miR-613 to regulate colon cancer growth and metastasis through targeting RTKN. Biomed Pharmacother 2019; 109: 2035-42.
23. Wang N, Zhong C, Fu M, et al. Long non-coding RNA HULC promotes the development of breast cancer through regulating LYPD1 expression by sponging miR-6754-5p. OncoTargets Ther 2019; 12: 10671.
24. Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic signaling pathways in the cancer genome atlas. Cell 2018; 173: 321-37.
25. Wong KK, Engelman JA, Cantley LC. Targeting the PI3K signaling pathway in cancer. Curr Opin Genetics Develop 2010; 20: 87-90.
26. Zhu Y, Zhang X, Qi L, et al. HULC long noncoding RNA silencing suppresses angiogenesis by regulating ESM-1 via the PI3K/Akt/mTOR signaling pathway in human gliomas. Oncotarget 2016; 7: 14429-40.