

IL-10 Promotes Glioma Cell Growth and Invasion via Upregulation of KPNA2 In vitro

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Abstract

Background Glioma is one of the leading causes of death worldwide with high incidence, recurrence and mortality. IL-10 is a cytokine with dual function in many types of tumors. Although IL-10 is overexpressed and promotes tumor progression in human primary brain tumor, the mechanisms are largely unknown. **Methods** Glioma cells were treated with different dosages of IL-10. The cell growth was detected by CCK8, and the invasion was measured by Transwell. The relative expression of mRNAs was detected by Quantitative real-time PCR (q-PCR). **Results** We found that IL-10 treatment significantly enhanced glioma cell growth and invasion. And KPNA2 was significantly upregulated after treatment with IL-10. By performing knockdown experiments, we found that the glioma cell growth and invasion were significantly declined. **Conclusions** The results indicated that knockdown of KPNA2 significantly inhibited the growth and invasion of glioma cells. And IL-10 promotes glioma progression via upregulation of KPNA2. This study will be of important significance and provides a potential target for treatment of patients with glioma.

Background

Glioma is the most common tumour arising from central nervous system (CNS) with an incidence of 22 per 100000 population [1], accounting for 75% of malignant primary brain tumours in adults [2]. It initiates from glial or precursor cells and the 5-year survival rates are around 10% [3]. Currently, the therapeutic strategies for glioma are surgical resection, combined with radiotherapy, chemotherapy, and/or targeted therapy according to the molecular features of the tumour for patients with newly diagnosed with glioma [4]. However, glioma remains incurable and its poor prognosis contributes to high rates of recurrence. Thus, it is essential to develop new strategies to inhibit glioma progression.

Interleukin-10 (IL-10) was first considered as a T helper 2 cytokine, which modulates the growth and differentiation of innate immune cells, endothelial cells and keratinocytes and suppresses the activation and functions of T cells [5]. Numerous kinds of cells can produce IL-10, such as Tregs, B cells, macrophages, mast cells, dendritic cells and epithelial cells in mice and human [6, 7]. IL-10 was regarded as a suppressive factor that impaired the proliferation, cytokine production and migration of effector T cells [8], elevated level of IL-10 inhibited cytolytic activity in transplanted tumors [9, 10]. In the context of tumour, IL-10 is a double edged sword. IL-10 administration suppressed tumor growth and led to tumour rejection in a variety of tumours, such as melanoma, sarcomas and colorectal cancer [11-14]. Conversely, IL-10 can also promote the development of lung cancer, esophageal squamous cell carcinoma, melanoma, cervical carcinoma [15-18]. In human brain tumour, IL-10 is overexpressed in human glioma, and its overexpression increases glioma cell proliferation and motility and promotes tumourigenesis [19-22]. However, the mechanisms of IL-10 on glioma development are not clearly understood.

KPNA2 is a member of Karyopherin α family which belongs to a family of nuclear transport proteins [23]. KPNA2 is composed of a N-terminal hydrophilic importin β -binding domain, a central hydrophobic region, and a short acidic C-terminus and functions through regulating the subcellular translocation of cancer

associated cargo proteins [24, 25]. It was reported that KPNA2 was overexpressed in a variety of types of cancer and promoted cell growth and survival, such as breast cancer, cervical cancer, colorectal cancer and esophageal squamous cell carcinoma [26-30]. In human brain tumour, KPNA2 is overexpressed in meningiomas and infiltrative astrocytomas, and correlates significantly with the histological grade and proliferative activity [31, 32]. In human glioblastoma, Lu et al. reported that MIR517C inhibited the epithelial-to-mesenchymal (-like) transition phenotype through KPNA2-dependent disruption of TP53 nuclear translocation, indicating KPNA2 is correlated with glioblastoma development [33]. Therefore, whether IL-10-mediated inhibition of glioma cell growth and motility is dependent on KPNA2 remains unclear.

In this study, we first evaluated the effect of IL-10 on glioma cell growth and invasion. Then, the expression of KPNA2 was quantified by qPCR and knocked down using short hairpin RNA (shRNA) to demonstrate the role of KPNA2 in glioma cell growth and invasion. This study will be of important significance and provides a potential target for treatment of patients with glioma.

Methods

Cell line and cell culture

Glioma cell line U87 was purchased from American Type Culture Collection (ATCC), and cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin G, and 100 µg/ml streptomycin at 37°C in a 5% CO₂ incubator. In all experiments, U87 cells were trypsinized at 80% confluency.

Cell proliferation assay

The CCK-8 assay was used to determine cell proliferation ability. In brief, cells were seeded at a density of 1×10^3 cells/well in 96-well plates and incubated overnight at 37°C. IL-10 was then added into the culture medium. at the indicated time points (1, 2, 3, and 4 days), 10 µl of CCK-8 solution was added into the culture medium, and the cells were incubated for an additional 1.5 hours at 37°C. Then, the absorbance of each well was measured at 450 nm using a microplate reader. All experiments were performed in quintuplicate.

Cell invasion assay

Put 100 µl diluted matrigel into upper chamber of 24-well invasion chamber and incubate at 37°C in a 5% CO₂ atmosphere for 4-6 hours to hydrate the matrigel. Then 500 µl serum-free medium was added into bottom well for 30 minutes. Cells were resuspended in 100µl serum-free media at a density of 10^6 cells/ml, then cell suspension was added into upper chamber and 500µl completed medium was added

into bottom well. After incubation overnight, cells on the upper surface of the filter membrane were scraped with cotton swabs, and those cells on the lower surface were fixed with polyoxymethylene for 30 minutes and stained with 0.1% crystal violet solution for 20 minutes. Five visual fields were randomly selected and photographed under a light microscope, and the invaded cells were counted.

RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

Total RNA from U87 cells was extracted purified using RNA extraction kit according to the manufacturer's protocol. Reverse transcription was performed to generate complementary DNA (cDNA) according to the manufacturer's protocol. The mRNA level of KPNA2 was measured by qPCR using SYBR premix Ex Tap. The primers were used as follows:

β -actin forward, 5'- CCGAGCCGTGTTTCCT -3';

β -actin reverse, 5'- GTCCCAGTTGGTGACGATGC-3';

kpna2 forward, 5'- TGATATGTCATCTTTAGCATGTGGC-3';

kpna2 reverse, 5'- GCCCACACAGCTTCCTTTTG -3';

β -actin was used as internal control. The relative mRNA expression of these genes were calculated using the $2^{-\Delta\Delta Cq}$ method [34].

siRNA transfection

The siRNAs were purchased from Ribobio company (Guangzhou, China), and the sequence of siRNA for KPNA2 is as below:

siKPNA2-1: ATTTACAGTGCCCTGGTTG;

siKPNA2-2: ATGAACGAATTGGCATGGT;

siKPNA2-3: GCATGTGGCTACTTACGTA.

U87 cells were seeded in 6-well plates and cultured overnight. Next day, cells were transfected with three siRNA for KPNA2 at optimal concentration. Forty-eight hours later, cells were used for further experiments.

Statistical analysis

The Student's *t*-test was used to quantify significant differences. GraphPad Prism 5 software was used for statistical analysis, Data are presented as the mean \pm SD, and a value of $P < 0.05$ were considered statistically significant.

Results

1. IL-10 promotes the proliferation of glioma cells

Cancer progression is a complex process involving cell growth, migration, invasion, colony formation, and metastasis. To explore whether IL-10 affected glioma progression, we first detected the role of IL-10 in glioma cell growth. We cocultured glioma cell line U87 with different dosages of IL-10. After 24 hours, CCK-8 assay was performed. We found that IL-10 promoted the cell growth of U87 in a dose-dependent manner (Figure 1A). As shown in Figure 1A, IL-10 at the concentration of 50 ng/ml enhanced the proliferation of U87 cells much significantly than others (Figure 1A). Therefore, we used IL-10 at 50 ng/ml for further experiments. We also found that IL-10 significantly promoted the cell growth of U87 cells at day4 compared with negative control cells (NC) (Figure 1B). Thus, these data demonstrated that IL-10 promotes the proliferation of glioma cells.

2. IL-10 enhances the invasion of glioma cells

Next, we assessed the role of IL-10 in glioma cell invasion by cell invasion assay. As compared with negative control cells, IL-10 treatment remarkably increases the number of invasion cells (Figure 2A, 2B). These results demonstrated that IL-10 treatment enhanced the invasion of human glioma cells.

3. IL-10 increases the expression of kpna2 in glioma cells

We further explored the mechanisms by which IL-10 promoted the glioma cell proliferation and invasion. Many studies have shown that KPNA2 played a role in cell growth and invasion of many cancer cells, including human brain tumour [26, 27, 29, 31, 32]. Therefore, we examined the expression of kpna2 in IL-10 treated U87 cells. We found that IL-10 significantly increased kpna2 mRNA levels in U87 cells compared with untreated control cells (Figure 3).

4. Knockdown of KPNA2 inhibits the proliferation of glioma cells

To investigate the role of KPNA2 in glioma cell growth, we used siRNA to specifically silence KPNA2 in U87 cells. We first examined the knockdown efficiency of KPNA2. As shown in Figure 4A, the expression

of KPNA2 was significantly inhibited in U87 cells. We found that knockdown of KPNA2 significantly inhibited the cell growth of U87 cells at day3 and day4 compared with negative control cells (NC) (Figure 4B). Taken together, these data demonstrated that KPNA2 affected the glioma cell growth.

5. Knockdown of KPNA2 suppressed the invasion of glioma cells

We also assessed the effect of KPNA2 on cell invasion of U87 cells. The results showed that knockdown of KPNA2 significantly decreased the cell invasion of U87 cells. Thus, we confirmed that kpna2 contributes the invasion of glioma cells.

Taken together, we found that IL-10 promoted glioma cell progress via upregulation of KPNA2.

Discussion

In this study, we report that IL-10 promotes cell growth and invasion in glioma cells. We found that IL-10 increased the expression of KPNA2, and knockdown of KPNA2 significantly inhibited the glioma cell growth and invasion, indicating that IL-10 promotes glioma cell growth and invasion via upregulation of KPNA2.

Immunotherapy for cancer is a new and promising treatment method besides operation, radiotherapy and chemotherapy. And it has been applied to treat malignancies, such as lung cancer, melanoma, renal carcinoma and hematological malignancy [35]. IL-10 is a cytokine which plays dual function in many types of cancers [11-18]. For example, IL-10 promotes resistance to apoptosis and metastatic potential in lung cancer cell lines [15]. Overexpression of IL-10 leads to frequent event of immune evasion in esophageal squamous cell carcinoma [17]. IL-10 facilitates the progression of cervical cancer [18]. However, IL-10 is also reported to inhibit tumor development, growth, and metastasis in melanoma, colon cancer and fibrosarcoma [12, 14]. Glioma is known to secrete IL-10 [36]. Increased production of IL-10 was also reported in glioma [36, 37]. In glioma, IL-10 stimulates the tumor-associated macrophages to express B7-H1, thus suppressing the immune system [38]. On the other hand, under the influence of glioma, microglia release several classes of molecules, including IL-10, that promote glioma growth, progression and inflammatory activation [39]. To clarify the function of IL-10 in human glioma progression, we detected the glioma cell growth and invasion after treatment with IL-10. Our results reveal that IL-10 treatment promotes the glioma cell growth and invasion.

Heterogeneity is a hallmark of cancer [40, 41]. Thus, the mechanisms of tumour cell growth, invasion, migration and metastasis in various types of tumour may be different and there are many factors to promote tumour progression in the same type of tumour. For example, long noncoding RNAs, circular RNAs, cytokines and migrating myeloid cells are all associated with glioma progression [19, 22, 42-44]. KPNA2 is a member of Karyopherin α family. It promotes tumour progression in many kinds of tumours, including glioma, breast cancer, cervical cancer, colorectal cancer and esophageal squamous cell

carcinoma [26-29, 31-33]. KPNA2 is required for IL-1 β -induced MMP-mediated metastasis in oral cavity squamous cell carcinoma [45]. KPNA2 also enhanced TNF α -induced expression of IL-6, MMP-1, and MMP-13 and increased the P65 phosphorylation in human synovial sarcoma cells [46]. Thus, whether KPNA2 mediates IL-10 induced glioma cell growth and invasion remains unclear. In our study, we found that KPNA2 was dramatically upregulated in glioma cells after treatment with IL-10. By using shRNA, knockdown of KPNA2 significantly inhibited glioma cell growth and invasion, indicating that IL-10 may promote glioma cell growth dependent on KPNA2. However, further experiments will be necessary to identify the interaction of IL-10 with KPNA2.

In view of a recent study showed that KPNA2 plays a crucial role in the metabolic reprogramming of glioma cells by mediating the nuclear transportation of c-Myc and E2F1 [30], we hypothesis that IL-10 may have an important role in regulating the metabolism of glioma cells, but further experiments are needed to prove it.

Conclusion

We confirmed that IL-10 promoted glioma cell growth and invasion, and we proposed for the first time that IL-10 promoted glioma cell growth and invasion via upregulation of KPNA2. Our study provided a potential target for treatment of patients with glioma.

Abbreviations

KPNA2: Karyopherin α 2

IL-10: Interleukin-10

CNS: central nervous system

q-PCR: Quantitative real-time PCR

shRNA: short hairpin RNA

Declarations

Ethics approval

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Tao Xin, Wei Sun and conceived the concept, designed the experiments. Zihao Liu and Jie Li wrote the manuscript. Zihao Liu, Xiaoming Huang, Haitao Fan, Fan Yang and Rui Zhang carried out and interpreted the experiments. Yihang Yang, Shaobin Feng, Dong He analyzed the data. All authors read and approved the final manuscript.

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Not applicable

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Figures

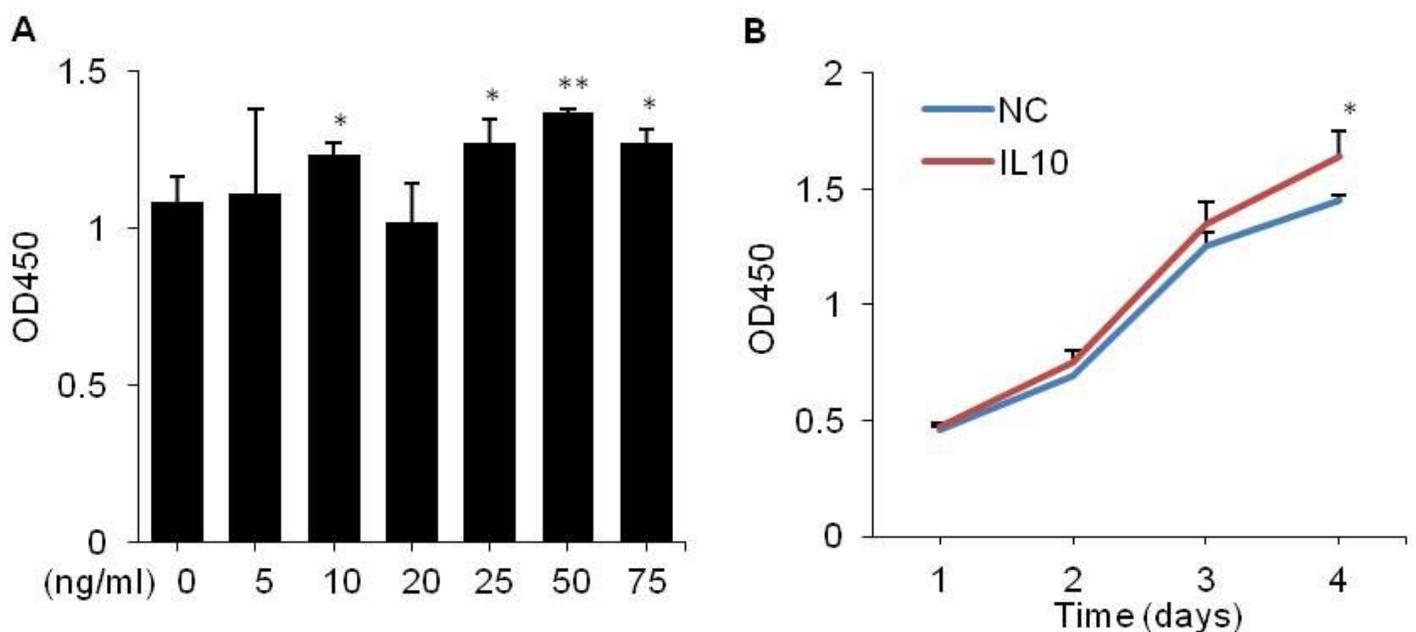


Figure 1

IL-10 enhances glioma cell proliferation - (A) U87 cells were cultured with different concentrations (5 ng/ml, 10 ng/ml, 20 ng/ml, 25ng/ml, 50 ng/ml and 75 ng/ml) of IL10 for 24 hours, then CCK8 assay was performed. The results were presented as mean±SD. * p<0.05, ** p<0.01. (B) U87 cells were treated with or without IL-10 (50 ng/ml) for 24 hours. Cells without treatment of IL10 were used as controls (NC). CCK8 assay was performed everyday for four days. The experiment was performed in quintuplicate. Data were shown as mean±SD. * p<0.05.

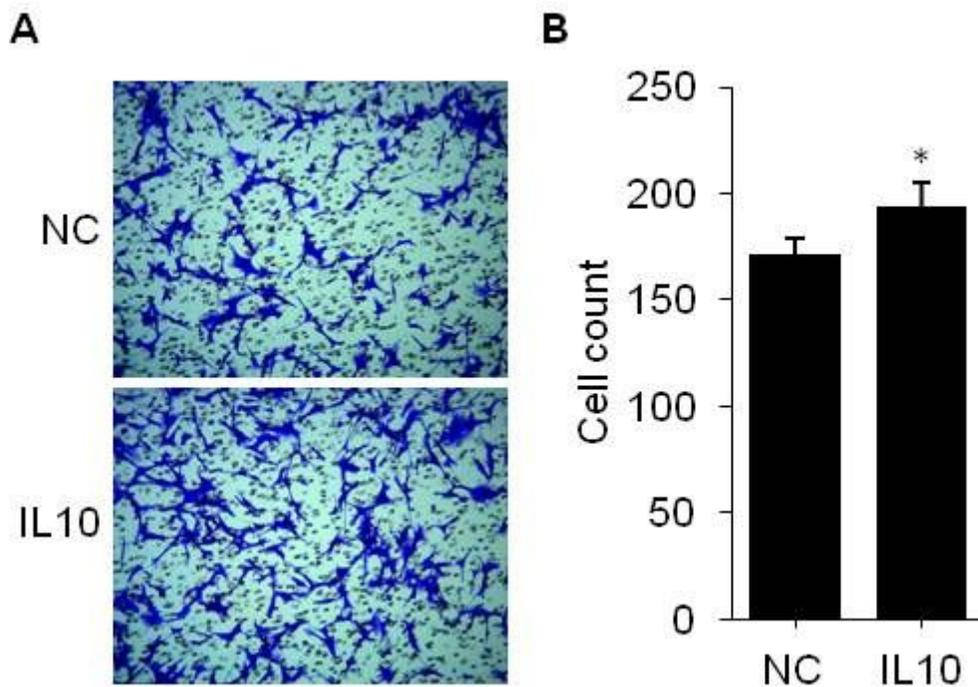


Figure 2

IL-10 promotes glioma cell invasion - (A-B) U87 cells treated with or without (NC) IL-10 were seeded in matrigel-coated Transwell chamber overnight. Cell invasion assay was performed (A) and invasion cells were counted from at least five random fields (B). Data are shown as mean ± SD. * p<0.05.

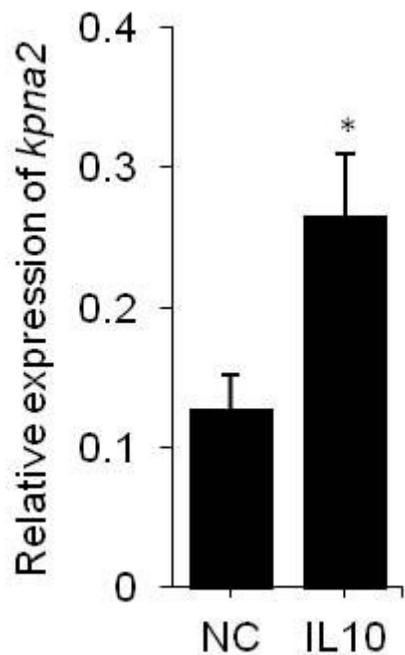


Figure 3

IL-10 increases the expression of KPNA2 in glioma cells - U87 cells were treated with or without (NC) IL-10 for 48 hours. The mRNA levels of *kpna2* was assessed by quantitative real-time PCR. Results shown are the mean \pm SD of three independent experiments. * $p < 0.05$.

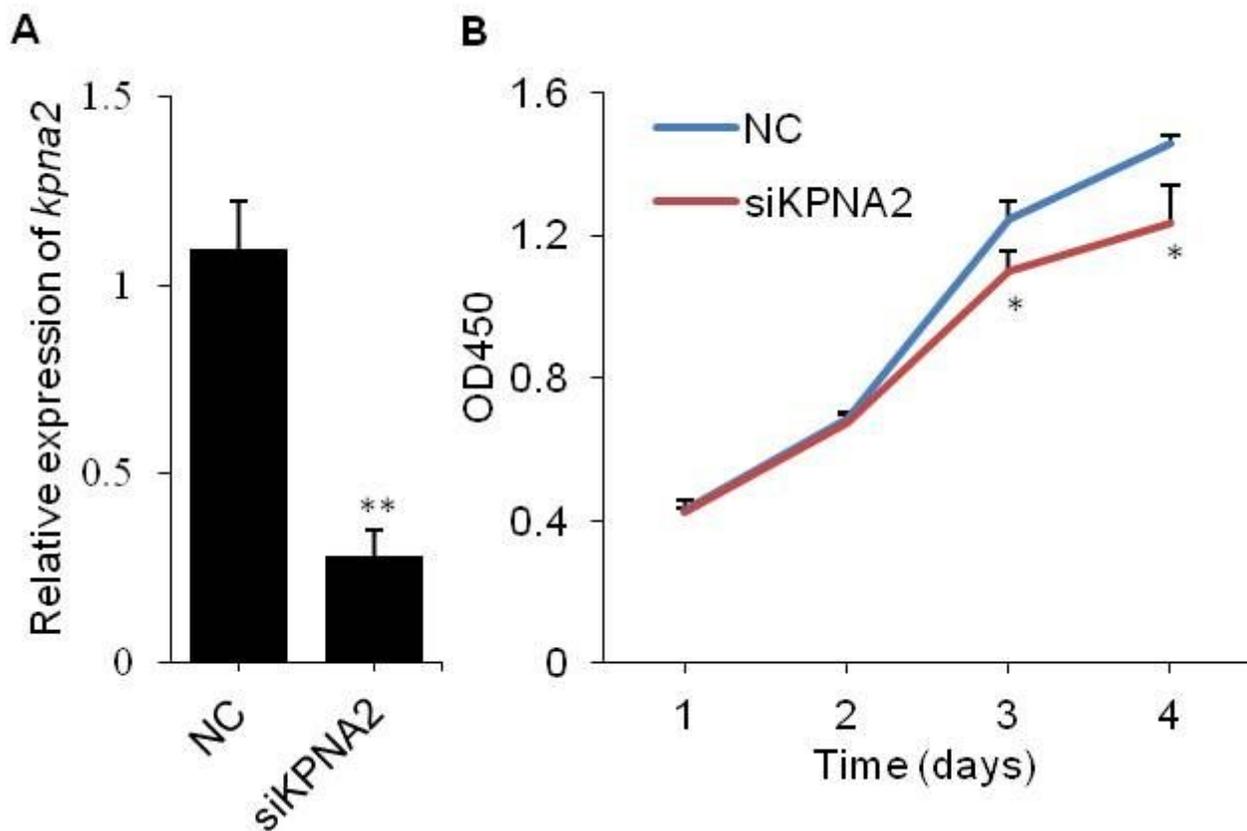


Figure 4

KPNA2 knockdown suppressed glioma cell growth - (A-B) U87 cells were infected with the negative control (NC) or KPNA2-shRNA lentivirus. (A) The mRNA levels of *kpna2* was assessed by using quantitative real-time PCR. Data are shown as mean \pm SD of three independent experiments. (B) CCK8 assay assessed cell proliferation every day for 4 days. The experiment was performed in quintuplicate. Data were shown as mean \pm SD. * $p < 0.05$.

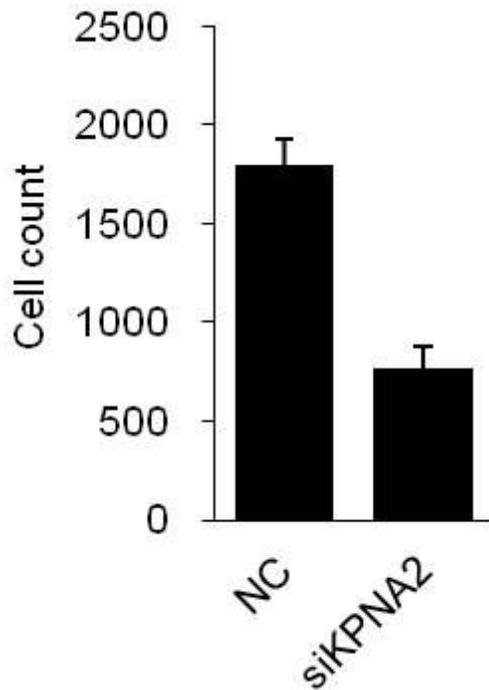


Figure 5

KPNA2 knockdown inhibited the glioma cell invasion - U87 cells infected with the control (NC) or KPNA2-shRNA lentivirus were seeded in matrigel-coated Transwell chamber overnight. Then, cell invasion assay was performed. Invasion cells were counted from at least five random fields. Data were presented as mean \pm SD. * $p < 0.05$.