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# MIRNA-223 Reduced Migration Activity and Proliferation of Nasopharynx Carcinoma Cells Through PI3K/AKT Signaling Pathway by Regulation Of IGF-1R

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**Abstract:**
The purpose of our study was to assess and confirm the roles of miRNA-223 expression in the prognosis and migration of nasopharynx carcinoma (NPC) cells, and to explore novel downstream targets. Our findings suggested that miRNA-223 expression in NPC tissues was lower compared to the para-carcinoma tissues. Additionally, downregulation of miRNA-223 expression promoted the proliferation and accelerated the apoptosis of 13-9B cells in a dose-dependent manner. Besides, downregulation of miRNA-223 expression reduced caspase-9 and caspase-3 expression levels, and downregulated Bax expression levels in 13-9B cells. Importantly, expressions of p-Akt1 (phosphorylation-Akt1) and PI3K in 13-9B cells were enhanced by downregulated miRNA-223 expression through activating IGF-1R (insulin-like growth factor 1). Moreover, the influence of miRNA-223 in the growth of 13-9B cells was reversed by activation of IGF-1R. In conclusion, miRNA-223 plays a role as a novel tumor suppressor in the development of NPC through PI3K/Akt signaling pathway by regulation of IGF-1R.

**Keyword :** miRNA-223, IGF-1R, p-Akt1, nasopharyngeal carcinoma

## Introduction

NPC (nasopharyngeal carcinoma) is special in the way that it involves all adolescents and the elderly, its origins, its history, professional behaviour and its disease therapies. In certain countries it is correlated with a low occurrence slightly below 1/100,000. The statistics indicate that NPC was 11th among the malignancies in China in 2008 (1), with an occurrence of 1,9/100,000 population a year and 2,8/100,000 for men and women respectively (2). In China, the highest incidence has been reported at 27.2/100.000 and 11.3/100,000 respectively in the towns of Sihui (Guangdong province), with incidence rates in men and women (3). Nasopharynx has a complex internal form, and a mass or lump to the back of the neck is a characteristic of NPC on both sides. 60-85 percent of NPC patients were stated to have regulation levels of NPC have dramatically improved clinically observed active metastases when diagnosed. Luckily, in recent years (3) the local thanks to developments in mri, radiation treatment, and the usage of combination chemotherapy (3). Nonetheless, the main cause of NPC treatment failure appears to be remote metastasis (4).

In addition to the coding genes and proteins linked to tumour, tumor metastases and growth (5) have also been documented to be strongly connected to the non-coding genes. In specific, miRNAs (microRNAs) can inhibit post-transcriptive gene expression, which is a non-coding of a single beach molecules with strongly conserved properties. In UTRs (3 ' untranslated regions), incomplete target complementarity is used for cognate mRNA, while translatory removal / degradation of the intended transcript is induced (6). More than 50% of miRNA genes have been detected in genomic regions or vulnerable sites linked with cancer (7). Structural alterations of the genome are also very popular in the human genome, for example variations in gene copy number, and miRNAs and copy numbers variants are novel organisms that have altered gene expression and their control (7). Multiple tumors that play a function in tumor suppressor genes or in

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anti-oncogenes may also cause aberrant miRNA expression (7). In almost all cases of cancer, irregular expression of miRNA is usually observed (7). Furthermore, previous research has shown that amiRNA expression profile may be critical for separating tissues from normal tissue from cancer of the head and neck, and thus contributes to molecular tumor classification (5).

Furthermore, a signal transduction route named the PI3K / Akt signal signal transport will improve cellular growth and survival while extracellular indications occur (8). P3 K (phosphatidylinositol 3-kinases) which belong to the lipid kinase and 4 forms of lipid products from the addition of a phosphate groups to the 3 "ring of PIs or Ptdins (9). The Akt signaling route is a signal transduction route that encourages survival and development while responding to extracellular signals. Akt is the basic key to signal transmission, where certain hormones, certain growth factors, nutry and cytokines produce extra-cellular signals that are capable to regulate a variety of cellular functions, including angiogenesis, digestion, self-reportedness, overvival, and proliferation, exocytosis, and motility (10).

Notably, IGF-1 (insulin-like growth factor 1) is a protein on the surface of the human cell, which is often active in several pathways for the development of tumor, mitosis. Specifically, tumors are used in focused treatment (12, 13) for chemo, radiotherapy, HER-2 and EGFR (epident receptor of growth factor) tolerance (12, 13). In several tumor cells, IGF-1 is over-expressed and repression of IGF-IR expression may reverse cell malignancy. In addition, for the transition activities of various oncogenes, the IGF-IR signal transduction mechanism is critical (14). Liu et al. found out that miRNA-223 in airways, with the inclusion of IGF-1R, suppresses extracellular matrix deposition in moist muscle cells (15). Earlier study has shown that miRNA can help colorectal cancer (16), bladder cancer pathogenesis (17) and breast cancer (18), doxorubicin resistance (16). This study analyzed the effect of miRNA-223 in the prognosis and migration of NPC, and investigated the novel downstream objectives.

**Subjects and Methods**

*Subjects.* The experiment was authorised by the Committee on Ethics of the General General Hospital of the People's Liberation Army (Beijing, China). The PLA's General Hospital (Bejing, China) hires NPC patients (n= 46) and better volunteers (n = 10). Centrifugation of serum samples at 1000 grams and 4 cents over twenty minutes was accomplished. The expressionmiRNA-223 in NPC patients were 0-0.2 of the miRNA-223 level as a limited level group in healthy volunteers; the expressionmiRNA-223 in NPC patients was 0.2-1 of miRNA-223, which was denoted as a large expression group in healthy volunteers (18). (21). The participants were tested at the tri-month clinics and the expression of miRNA-223 was analyzed with Kaplan-Meier's DFS (disruptive survival) and OS (total survival) study.

*Cell Culture.* Tumor cell line (Human NPC 13-9B) was given to DMEMs comprising FBS (fetal bovine serum at 10 percent; Gibco, New York, America at 30 centigrade in the presence of carbon (5 percent) by Cell Bank of the Form Culture Array of the Chinese Accounts of Science (located in Shanghai, China), and cultivated with DMEM (supported by Hyclone Laboratories, Logan, America).

*Speech examine miRNA-223. TRIzol reagent (supplied by Invitrogen, Carlsbad, USA) was used to remove complete RNA from NPC serum samples according to manufacturer instructions. The synthesis of cDNA (supplied by Applied Biosystems, Foster City, America) was also conducted by the Oligo[dT] cDNA Synthesis Pack.*

The cDNA has been diluted to 10 and five µL (100 ng) of cDNA have been extracted from each sample. We did quantitative PCR in real-time and SYBR Green was employed with Applied Biosystems, Foster City, America, as a tool for detecting input from Control SYBR Green PCR Master Blend. The parameters of the reaction: 30 seconds at 950 degrees Celsius, 40 intervals at 15 seconds at 95 degrees Celsius and 30 seconds at 60 degrees Celsius. A comparative approach was used to assess comparatively low gene expression.

Transfection with miRNA-223 and inhibitor of PI3K. Shanghai GenePharma Co., Ltd. (Shanghai, China) produced human miRNA-223 imitations, anti-miRNA-223 imics, and derogatory imics. Then, 13-9B was cultivated and transfected with unfavorable photos and anti-miRNA223 and Lipofectamine 2000 (provided with Invitrogen, Carlsbad, USA). Lipofectamines 2000 was also used. The cell line was also cultivated and transfected.

MTT exam. MTT check. Following 48 h of transfection, 13-9B cells were developed on 96-well plate and MTT (3-(4,5-dimethythiazol-2-yl)-2,5-di-phenyltetrazolium bromide) given to the Beyotime Biotechnology Institute were used for analysis of cell development. The cell was then transfected with or treated with a MINA-223 inhibitor. Four hours was incubated in thirty-seven degrees and DMSO (dimethyl sulfoxide), 150 µl was applied to kill formazan crystals in thirty-seven degrees, and 492 nm (Bio-Rad Laboratories Inc., Hercules,

America) was introduced to measure the absorption by the microplate reader in the textbook. as follows...

Flow cytometry. The flow cytometry procedure was used for determinating the apoptosis concentrations after 48 h of transfecting and inserting the cell into a 6-well dish. Flow cytonometry on BD FACS CaliburTM Flow Cytometers (provided by the BD Biosciences, San Jose, America) as per manufacturer's manual was performed after dark stained Annexin-V (10 μL; 1 μM) and PI (5 μL;5 μM) for half an hour.

*ELISA (enzyme-linked immunosorbent assay) assay.* The commercial ELISA kits were used for the identification of the caspase 3/9 cell response after the cell was put in 6-well plates. The Colorimetric Microplate Reader used to detect absorption at wavelengths of 405 nm after incubation of 13-9B-cells utilizing ac-LEHD-pNA (caspase 9) and Ac-DEVD-pNA (caspase 3) for two hours at a grade of thirty-seven centigrade (Bio-Rad Laboratories Inc., Hercules, America).

*Western blotting.* After transfection of 13-9B cells with miRNA-223 or treatment with PI3K inhibitor, the cells were added into a 6-well plate, followed by lysis for half an hour at four centigrade degree. Total amount of protein was detected by BCA (bicinchoninic acid) assay (Beyotime Institute of Biotechnology, Shanghai, China), and separated with 8-12% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), followed by PVDF (polyvinylidene fluoride) membrane (provided by Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom) transfer. After blocking in skim milk powder (5%) in TBST, membranes were subjected to incubation with the primary antibodies below at four centigrade degree overnight: Bax, Bcl-2, PI3K, p-Akt1, Akt, and GAPDH (glyceraldehyde 3-phosphate dehydrogenase), which were all purchased from Santa Cruz Biotechnology, Inc., Dallas, America. Membranes were subjected to incubation with anti-rabbit IgG secondary antibody by SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, America). The intensity of each band was quantified via ImageJ 3.0 software.

*Luciferase reporter assay*. In this case 13-9B cells were transfected using the reagent of Lipofectamine 2000 (bought from Invitrogen, Carlsbad, USA) in the 100 ng pGL3-IGF-1R-luciferase plasmid and miraRNA-223 mimics. The Reporters Assay Package, obtained from Promega, Madison, Americas, was used to assess luciferase activity after a 40-eight-hour transfection.*Statistics.* The data where represented in mean ± default (SD). The Kaplan-Meier estimator was used to evaluate the OS and the DFS. The ANOVA (oneway variance research) or t-test student has been developed for differences study. An important difference was stated by $P < 0.05$.

**Results**

NPC patients with MiRNA-223 amounts. A ChIP-qPCR (chromatin immunoprecipitation coupled with quantitative polymerase chain reaction) was used to detect miRNA-223 levels in NPC tissues and normal tissues. The findings revealed that the miRNA-223 level was considerably decreased in NPC tissues in contrast to standard tissue (Fig.1A-1B). OS and DFS were both shorter than NPC patients with a low miRNA-223 level with a strong miRNA-223 level (Fig. 1C-1D).

The proliferation of 13-9B cells has been controlled by MiRNA-223. The function of miRNA-223 in the proliferation and apoptosis of 13-9B calls is determined by transferring miRNA-223 or Anti-mi RNA-223 mimics to 13-9B cells that significantly could upregulate or disregulate miRNA-223 levels in 13-9B cells (Fig.2A-2B). In addition, overexpression miRNA-223 inhibited the migration function and proliferation of 13-9B cells (fig. 2C-2 G). Downregulation of miRNA-223 also greatly supported the movement and proliferation of cells of 13-9B (Fig.2H-2 K).

Apoptosis of 13-9B cells was induced by MiRNA-223. The role of miRNA-223 in 13-9B cells apoptosis was also studied. The findings show that cell apoptosis was decreased due to miRNA-223 overexpression, with increased lactate dehydrogenase (LDH) activity and Caspase-3/9 in 13-9B cells (Fig.3A-3E). The cell apoptotesis has, however, been decreased by downregulation of miRNA-223 and caspase-3/9 and LDH activation have been removed in 13 to 9 B cells ( Fig. 3F-3J).

Via the IGF-1R receptor, MiRNA-223 controlled 13-9B cells' proliferation through a PI3K/Akt signaling path. The activation of the IGF-1R / PI3K / Akt signal pathway was investigated by west blotting to demonstrate the fundamental process through which miRNA-223 controls the appoptosis of 13-9B cells. The ChIP-qPCR findings showed that miRNA-223 downregulation induced IGF-1R expression, while PI3 K expression was suppressed in 13-9B cells (Fig.4A-4C). As seen in the Fig. 4D-4E, miRNA-223 decomposite guide the IGF-1R 3'-UTR and improved luciferasis reporter gene expression.

Immunofluorescence has demonstrated, compared with negative control groups, that downregulation of miRNA-223 has resulted in IGF-1R expression in 13-9B cells (Fig. 4F). As seen in the

photo. The miRNA-223 overexpression inhibited 5A-5D, p-Akt, IGF-1R, and PI3 K signals in 13-9B cells. In comparison, p-act. Downregulation of miRNA-223 (Fig. 5E-5H), IGF-1R and PI3 K proteins in 13-9B cells are elevated.

Activation of the IGF-1R has decreased the impact of miRNA-223 on cell growth of 13-9B. IGF-1R plasmid was used to upright IGF-1R expression levels in 13-9B cells in order to gain insight into the possible pathways that may be involved in the modulation of miRNA-223. Results showed that the IGF-1R expression level was modified in cells 13-9B compared with the over-expressed miRNA-223 community (Figure 6A, 6D). In addition, activation of the IGF-1R mediated p-Akt and PI3 K receptors in the 13-9B cells by miRNA-223 upregulation in relation to myRNA-223 upregulation (Fig. 6B-6D). In addition, the activation of IGF-1R stimulated the migration and development of 13-9B cells, thus growing the apoptosis, as well as of LDH activity and caspase-3/9, via the up-regulation of miRNA-223 cells (Fig. 6E-6N).

**Discussion**

The miRNA linked to cancer is more conserved than non-cancer miRNA, which suggests lower likelihood that one nucleotide polymorphism (SNP) will occur (19). In the meanwhile, the number of cancers with miRNA is optimistic with their protective properties (20). Genomic position and sequence review found that miRNAs connected with cancer are typically present at non-cancer MIRNA clusters. Further study of the host genes, miRNAs related to cancer and goal genes has shown that host genes for certain NRNAs related to cancer are vulnerable to the miRNAs (7). We provide theoretical basis for a detailed study of the association between miRNA and cancer by utilizing miRNA as a diagnostic test for cancer. Our study has shown that miRNA-223 expression is apparently decreased relative to control tissues in NPC tissues. In comparison, the operating systems and the DFS were shorter for those with low miRNA-223 than for those that had high miRNA-223. In addition, miRNA-223 downregulation facilitated the proliferation and migration of human NPC cells. MiRNA has been shown to encourage colorectal malignancies tolerance (16), cyst malignancies (17), and breast malignancies (18) to doxorubicin. 13-9B cells were only included in our study for the vitro simulation of the NPC, a drawback. Many animal models and cell lines will also be tested in the future.

PI3K/PTEN/Akt signal transduction pathway can regulate growth of several tumors (21). The mechanism for the transduction of the signal PI3K / PTEN / Akt plays an essential role in apoptosis control. Activation of the signal transduction pathway PI3K / PTEN / Akt will inhibit apoptosis triggered by different stimuli, thus facilitating cell survival and cell proliferation, and encourage the progression of the cell cycle (21). Furthermore, it takes part in angiogenesis and is central to the development of tumours, metastases and invasions (23, 24).

This mechanism also affects malignant tumors' growth and metastasis by causing proliferation and angiogenesis of tumor cells (23,25). The present study shows that downregulation of miRNA-223 in 13-9B cells may induce IGF-1R, p-Akt, and PI3 K expression. Huang et al. note that miRNA-22, via PI3K / Akt signaling pathway controls, controlled proliferation of Glioblastoma stem cells and the chemoresistance to temozolomide (26).

Furthermore, IGF-1R affects migration, proliferation, and hypoxia reaction of tumor cells. Specifically, IGF-1R has been treated as a target within 20 years (27, 28), and it mainly plays its function through IGF-1R. Alternatively, it can bind to the IGF-1R-insulin receptor (IR) complex, so as to further activate downstream signaling, which is particularly confirmed for tumor cells. The IGF-1R signaling pathway can bind with other growth factors, which benefits the initiation of tumor metastasis and development (27, 29). Besides, it can activate PI3K/Akt and Ras/Raf/MAPK pathways through IGF-1R, thereby transmitting survival signals (27, 29). In the current study, it was revealed that activation of IGF-1R attenuated miRNA-223's influence in human NPC cell growth. Other study revealed that miRNA-223 inhibited the deposition of extracellular matrix in airway smooth muscle cells through the PI3K/Akt signaling pathway by targeting IGF-1R (15). The present research only used IGF-1R plasmid to increase IGF-1R expression levels in an in vitro model following overexpression of miR-223, which was herein found insufficient. We will use further Akt and IGF-1R inhibitors to regulate Akt or IGF-1R in the next research.

Conversely, miRNA-223 outright regulators also facilitated the migration and proliferation by controlling the IGF-1R of 13-9B cells through the PI3K / Akt signalling pathway. Our data together indicate that miRNA-223 guarantees care to patients with NPC

**Figure Legends**

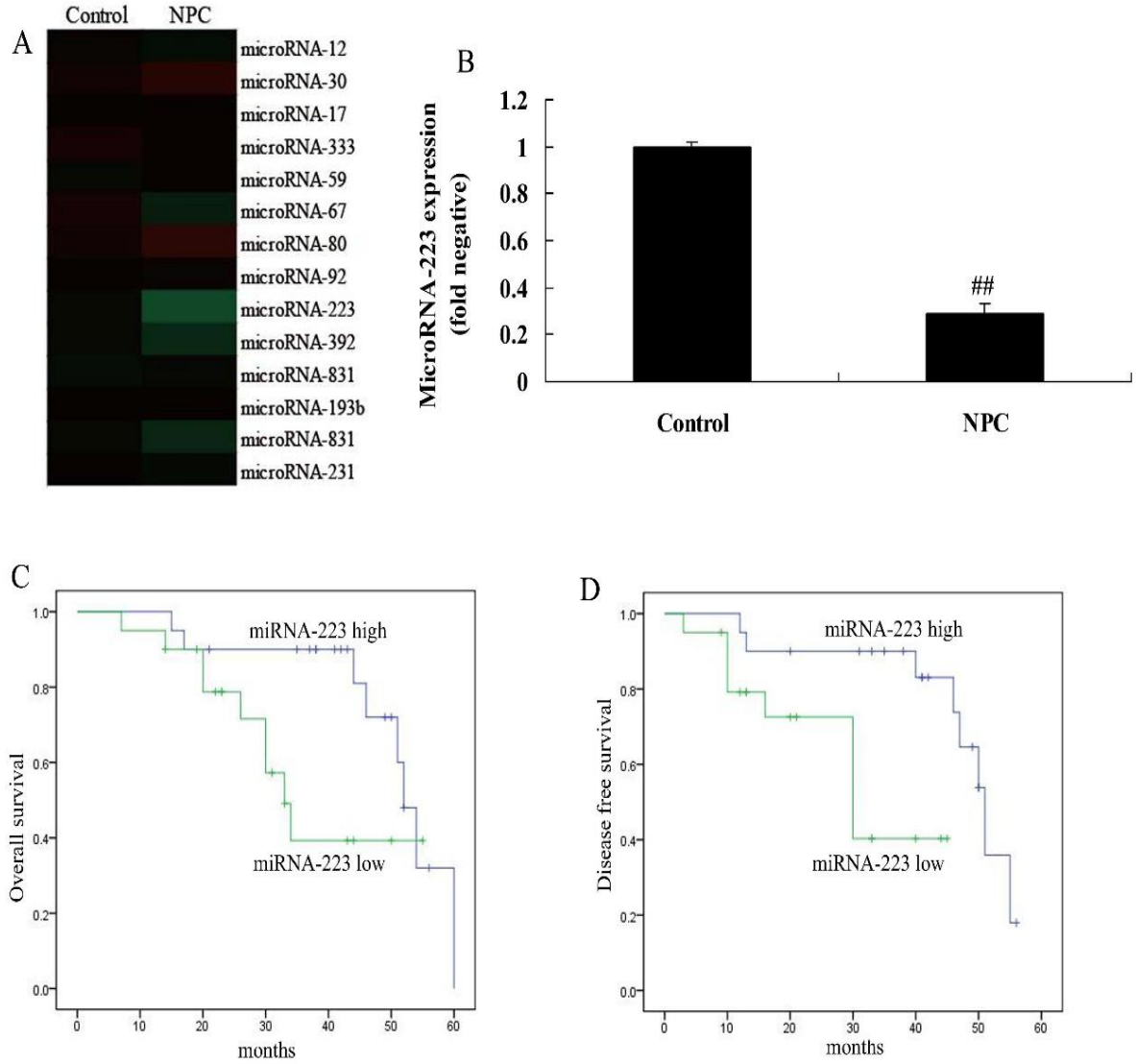


Figure 1. **The expression of miRNA-223 in NPC patients**

**In the NPC patient category, A and B as well as OS and DFS (C and D), ChIP-qPCR was used to identify miRNA-223. # P<0.01, relative to the NPC party. Usual, regular group; NPC group, NPC group of patients.**

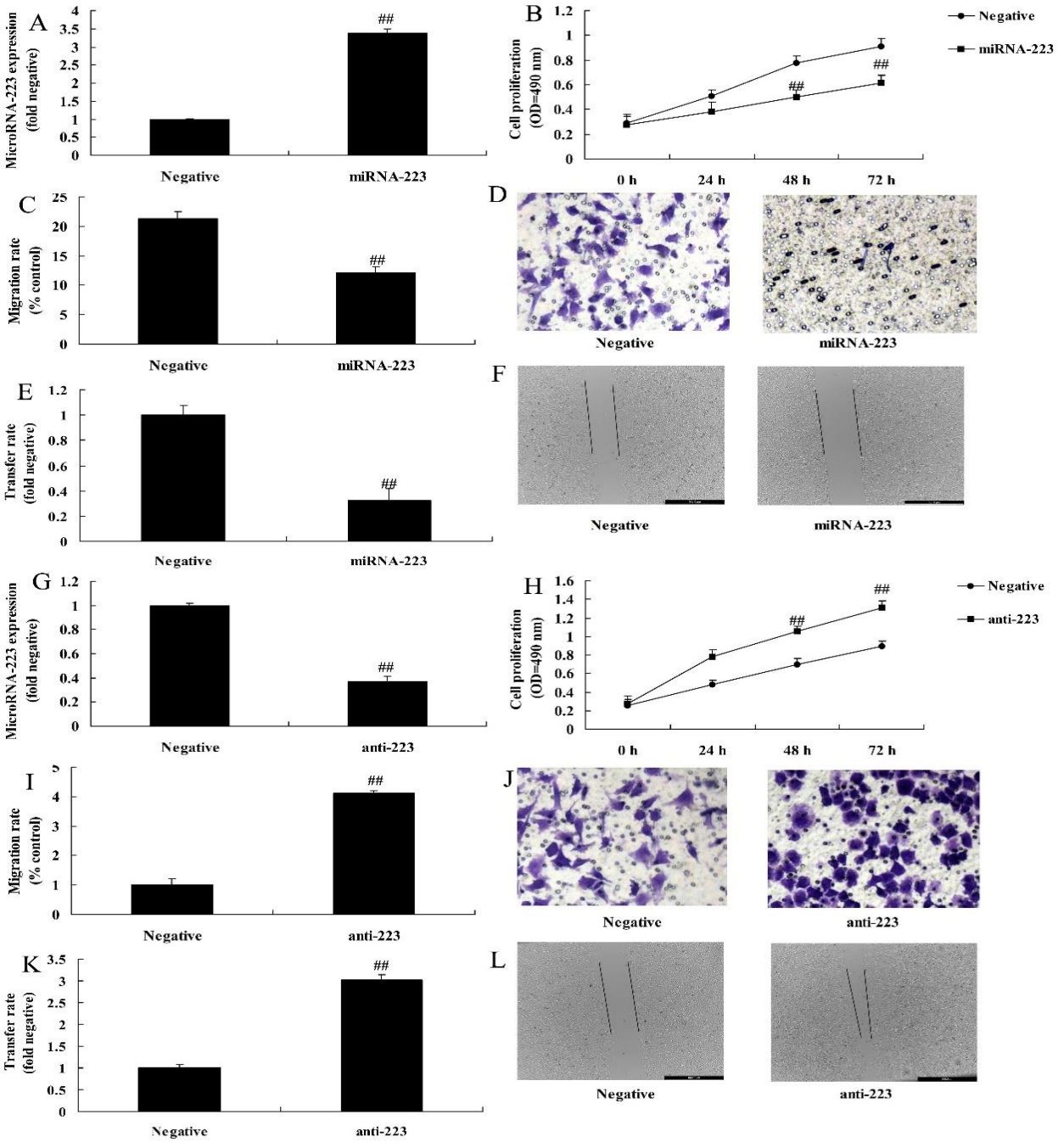


Figure 2. **Proliferation of 13-9B cells is regulated by MiRNA-223.**
**Expression miRNA-223 (A), cell proliferation (B), migration rates (C and D), transition rates (E and F) with over-expression miRNA-223; expression miRNA-223 (G) with proliferation of the cells (H), migration (I and J); transference rates (K and L) with a decrease in miRNA-223 amounts. miRNA-223 group, miRNA-223 level increases; dissatisfaction, monitor negative; anti-223 group; miRNA-223 level decreases. # # P<0.01, contrasted with the control group negative.**

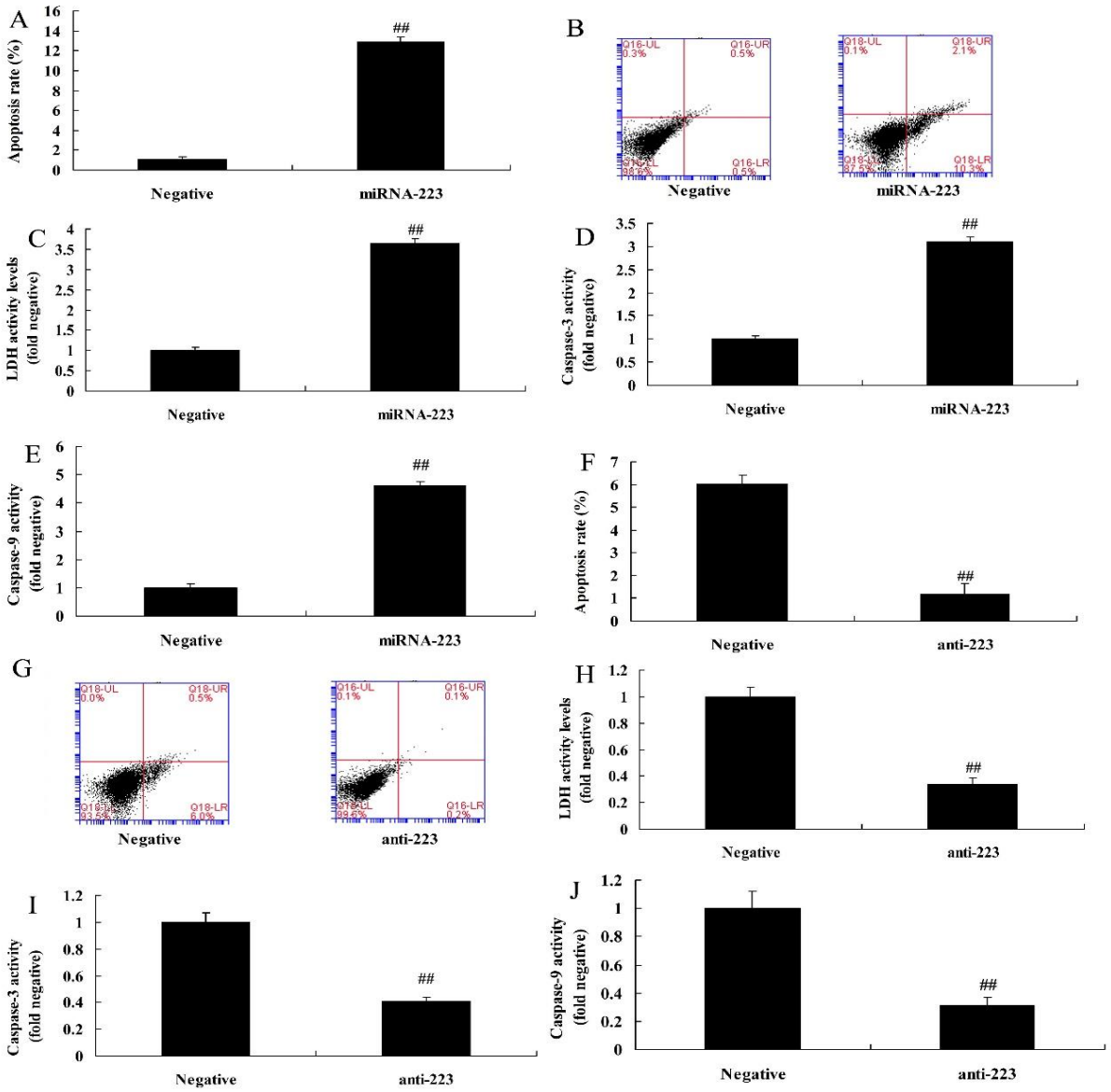


Figure 3. **MiRNA-223 regulates apoptosis rates of 13-9B cells.**

**The overexpression of the MIRRNA-223 was measured at apoptosis rates (A and B), the LDH (C, caspase-3 and caspase-9 levels (D and E); the apoptosis rates (F and G), LDH activity (H) and the MIRNA-223 reduction in Caspase-3 and Caspase-9 (I and J) were examined. The number of negative classes is # P<0,01. Bad group, negative group control; miRNA-223 group, miRNA-223 over-expression; miRNA-223 group, downregulation.**

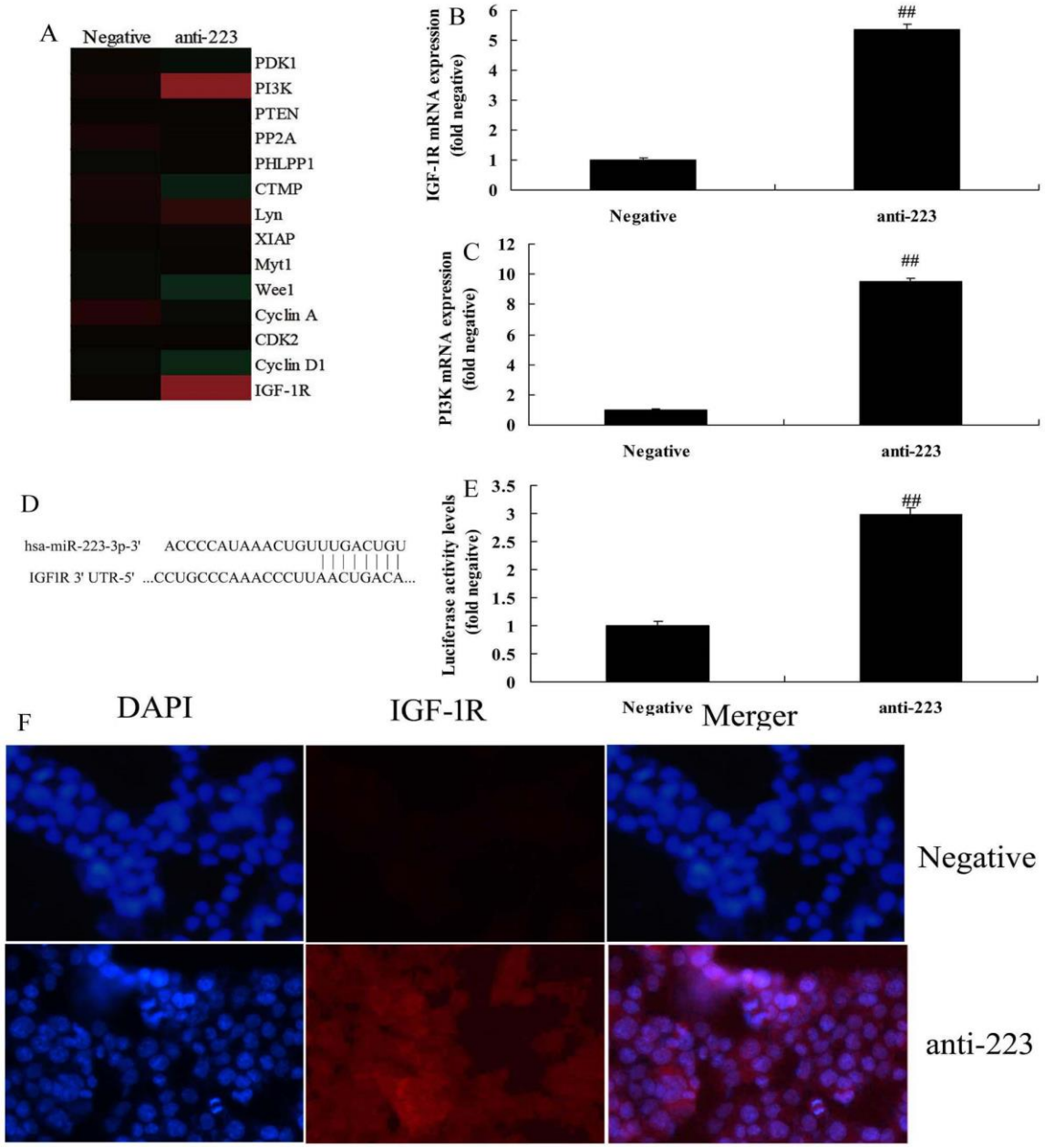


Figure 4. **MiRNA-223 regulates IGF-1R in 13-9B cells**

**ChIP-qPCR is used to evaluate IGF-1R and PI3 K (A, B and C) expression stages. The decline of miRNA-223 could be targeted by immunofluorescence directly to IGF-1R (D), luciferase activity study (E) 3'-UTR and IGF-1R expression levels (F). # # P<0.01, relative to negative community power. Negative group; negative community control; anti-223 community, miRNA-223 downregulation.**

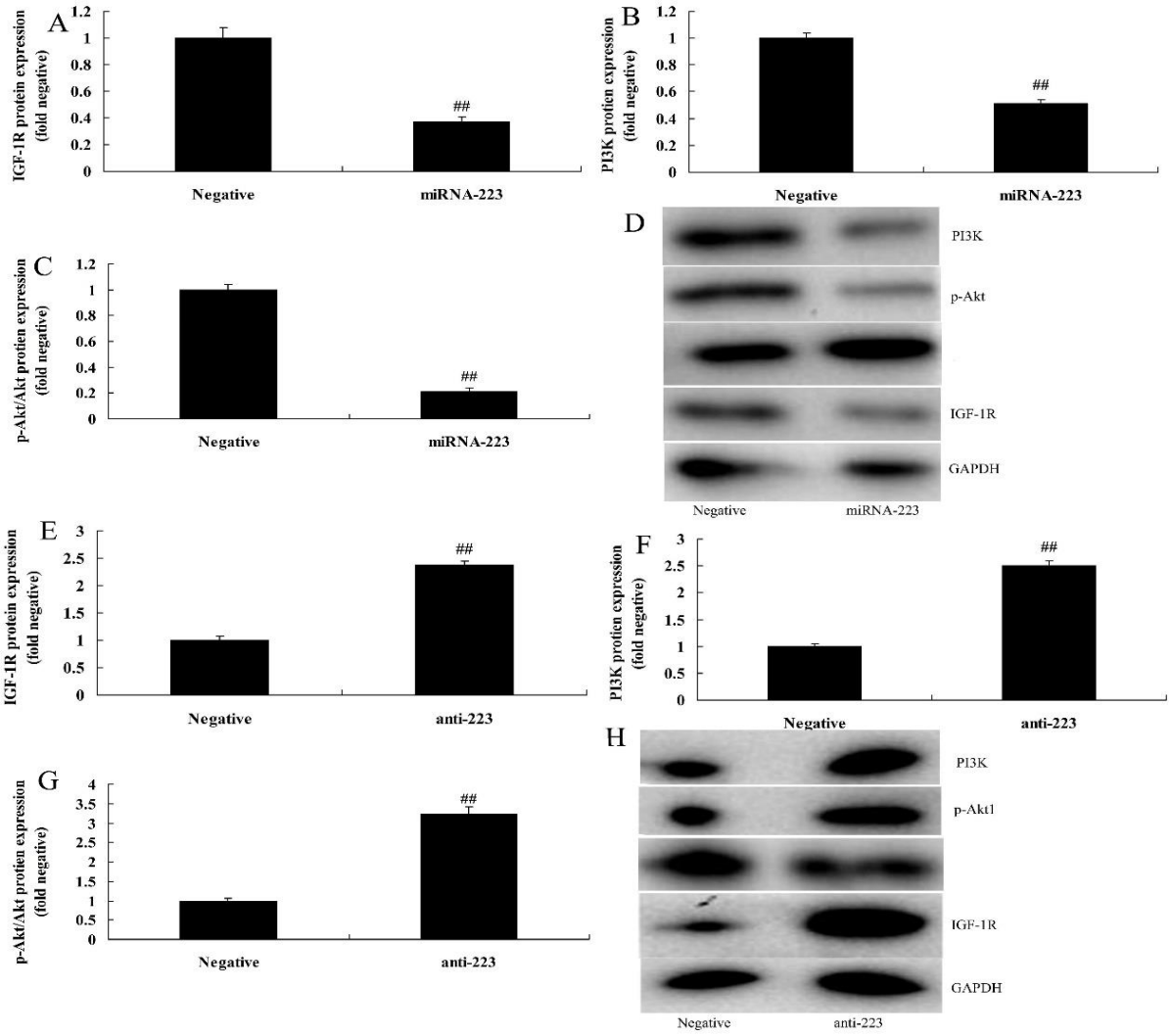


Figure 5. **MiRNA-223 regulates PI3K/Akt signaling pathway in 13-9B cells by targeting IGF-1R. PI3 K, p-Akt, and IGF-1R expression levels were evaluated by statistical (A, B and C) and western (D) over-expression from miR NA-223. Statistical analyses (E, F, & G) and western blotting (H) were used for the PI3 K, p-Akt, and IGF-1R expression to evaluate miRNA-223 expression level. negative group; miRNA-223 group, miRNA-220 levels increased; anti-223 group, miRNA-223 levels decreased; Group unfavorable; # P<0.01, relative to the negative community power.**

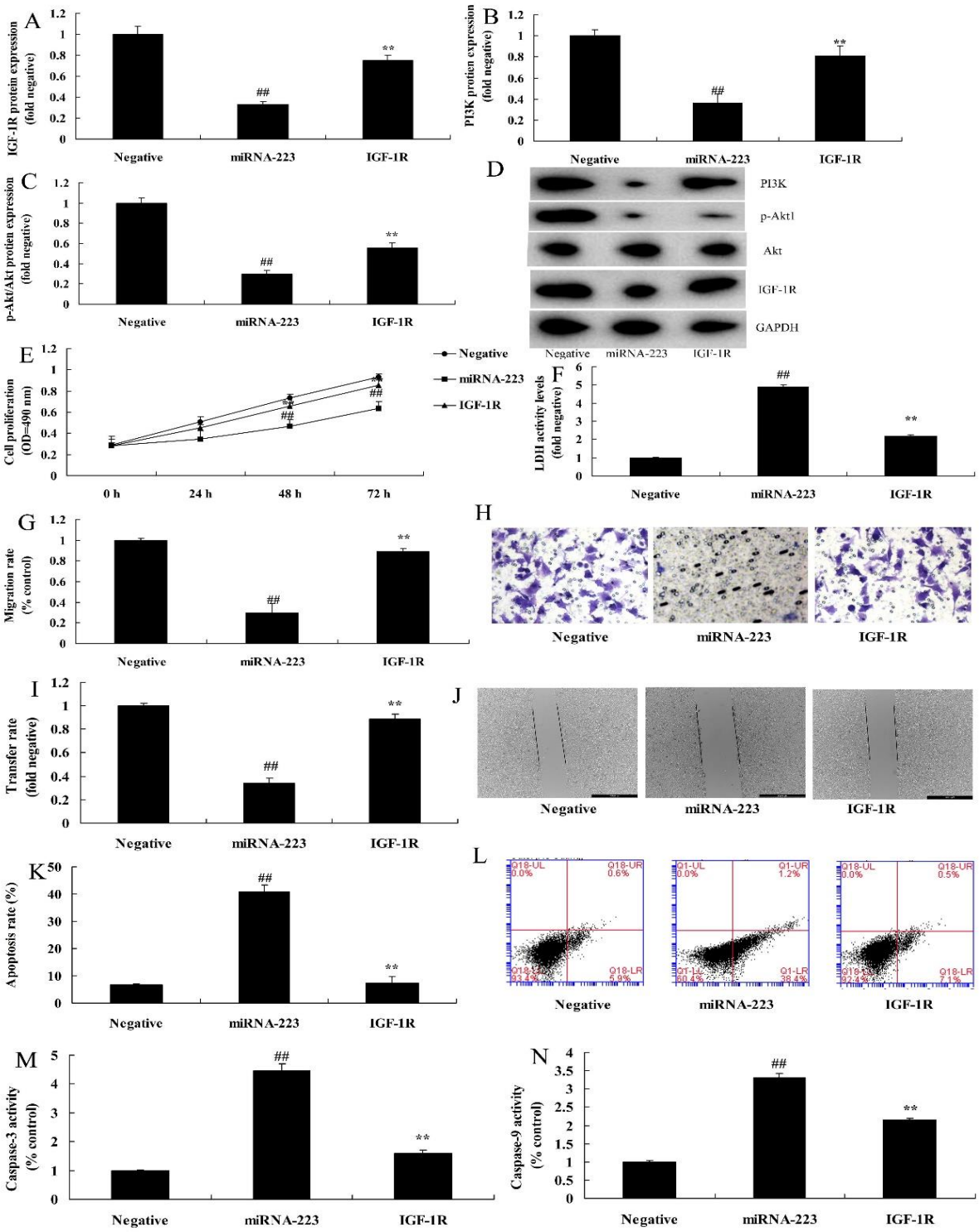


Figure 6. **The influence of miRNA-223 in growth of 13-9B cells was reversed by IGF-1R activation. Statistical analyzes (A, B and C) and Western blotting (D) evaluated the expressional quantities of PI3 K, p-Akt and IGF-1R. Proliferation cell (E), LDH (F), migration (G) and H), transition (I and J) and apoptosis (K and L), as well as caspase-3 and caspase-9 (M and N) levels were calculated. * * P<0.01 relative to miRNA-223 over-expression # # P<0.01, according to negative screening. Negative group, optimistic control group; miRNA-223 group, miRNA-223 over speech; IGF-1R group, IGF-1R activation and miRNA-223 over language.**