**MicroRNA-122 Promotes Proliferation, Invasion and Migration of Renal Cell Carcinoma Cells Through the PI3K/Akt Signaling Pathway**

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**Abstract**

Objective: MicroRNAs (miRNAs) are a small class of non-coding, single-stranded RNAs with a critical role in genesis and maintenance of renal cancer mainly through binding to 3'-untranslated regions (3'UTR) of target mRNAs, which causes a block of translation and/or mRNA degradation. The aim of the present study was to investigate the potential effects of miR-122 in human renal cell carcinomas.

Methods: The expression level of miR-122 was quantified by qRT-PCR. MTT, colony formation, invasion and migration assays were used to explore the potential functions of miR-122 in human renal cell carcinoma cells.

Results: Cellular growth, invasion and migration in two A498 and 786-O cells were significantly increased after miR-122 transfection. Further experiments demonstrated that overexpression of miR-122 resulted in the increase of phospho-Akt (Ser473) and phospho-mTOR (Ser2448), then activation of mTOR targets, p70S6K and 4E-BP1.

Conclusions: The up-regulation of miR-122 may play an important role in the progress of renal cancer through activating PI3K/Akt signal pathway and could be a potential molecular target for anti-cancer therapeutics.

**Keywords:** miR-122 - renal cell carcinoma - invasion - migration
transfected with Pre-miR miRNA precursor-122, anti-miR-122 or negative control precursor miRNA using LipofectamineTM 2000 Transfection Reagent (Life Technologies) following 24 h of cell seeding. Briefly, Pre-miR miRNA precursor-122, anti-miR-122 or negative control precursor miRNA and LipofectamineTM 2000 were diluted to 100 μL by RPMI 1640 medium without serum and antibiotics, respectively and incubated for 5 min. Then miRNAs-LipofectamineTM 2000 complex were gently mixed for 20 min at room temperature and added to the 6-well plates. After 6 h of incubation, culture medium was replaced by RPMI 1640 medium with 10% FBS, and the cells were incubated at 37°C in a CO₂ incubator for 48 h prior to further testing.

Cell proliferation assay (MTT)

Cell proliferation was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay performed according to the manufacturer’s instructions. Cells (5,000/well) with various concentrations of miR-122 mimics or negative control miRNA were seeded onto 96-well plates in triplicate and incubated for 72 h. MTT reagent was added and incubated for 4 hours. Then the formazan precipitate was dissolved in 150 μL DMSO and the absorbance rate was measured in a microplate reader at a wavelength of 570 nm. After 48 h transfection, cells were seeded in 24-well plates in triplicate at a density of 1×10⁴ cells per well for growth curve assay. The numbers of viable cell from control negative miRNA, miR-122 and anti-miR-122 transfected groups were counted at 24, 48 and 72 h.

Colony formation assay

For colony formation assay, cells were transfected with miR-122 mimics, anti-miR-122 or negative control miRNA for 48 h, and then grown in a 6-well plate in triplicate. After 14 days incubation, the colonies were gently washed with PBS and then fixed with 10% formalin and stained with 0.1% crystal violet for 30 min. The colony containing more than 50 cells was manually counted.

Invasion and migration assays

The invasion and migration assays were performed using the transwell system (24 wells, 8-μm pore size, BD Bioscience, USA). After 48 h transfection with miR-122 or negative control miRNA, 1×10⁵ cells were resuspended in RPMI-1640 medium without FBS and seeded into the upper chambers coated with growth factor reduced Matrigel (BD Bioscience) for invasion assay and collagen IV for migration assay and the lower chamber was filled with 0.4 ml of RPMI 1640 medium containing 10% FBS. Cells were fixed with 10% formalin and stained with 0.1% crystal violet solution after they were incubated for 24 h. Cells on the upper side of the membrane were removed by using a cotton swab. The invaded or migrated cells in triplicate were taken pictures and counted in five randomly fields under a light microscope at ×100 magnification for statistical analysis.
We further detected the influence of miR-122 on cell carcinoma cells

miR-122 enhanced invasion and migration of renal cell carcinoma cells

To study the effects of miR-122 mimics on the proliferation of RCC cells, A498 and 786-O cells were exposed to different concentrations of miR-122 mimics for 72 hours by the MTT assay. Our results showed that a significant increase in cell proliferation of A498 and 786-O in a dose-dependent manner was observed (Figure 2A, \( P < 0.05 \), \( ** P < 0.01 \), \( *** P < 0.001 \)) following miR-122 transfection, compared to cells transfected with miR-Con. For colony formation assay, our results showed that the colony numbers were significantly increased following miR-122 mimics transfection and decreased following anti-miR-122 transfection compared with the control cells (Figure 2B, \( ** P < 0.01 \), \( *** P < 0.001 \)). We also observed that the effect of miR-122 and anti-miR-122 on the growth rate of both cell lines by counting cell numbers in triplicate wells every day for 3 days. The growth of A498 and 786-O was significantly increased by miR-122 and decreased by anti-miR-122 in a time dependent manner (Figure 2C). These results indicated that renal cell carcinoma cell proliferation could be significantly increased by miR-122 and decreased by anti-miR-122.

miRNA-122 expression in different renal cell carcinoma cells

We examined a series of human renal cell carcinoma cell lines, A498, ACHN, Caki-1, Caki-2 and 786-O for miR-122 expression to choose appropriate renal cancer cell models. A498 and 786-O cells have very low expression levels of miR-122, but high levels of miR-122 in other cell lines (Figure 1A). Based on these data and our observation, we chose the two cell lines, A498 and 786-

Results

Expression levels of miR-122 in different renal cell carcinoma cells

We further detected the influence of miR-122 on cell
compared to control groups. All of these results suggest that up-regulation of miR-122 may play roles in the occurrence and development of RCC.

Recent studies found that miR-122 is frequently suppressed in hepatocellular carcinomas (Bai et al., 2009; Tsai et al., 2012) and breast cancer specimens (Wang et al., 2012). Overexpression of miR-122 could suppress proliferation and induce apoptosis and cell cycle arrest in cancer cells through reducing the expression of Bcl-W, CCNG1 (Ma et al., 2010), IGF1R/PI3K/Akt (Wang et al., 2012) and Wnt/beta-catenin signal pathways (Xu et al., 2012). These findings suggest that miR-122 may behave as a tumor suppressor in hepatocellular carcinoma and breast cancers. In contrast to what has been observed in the liver and breast cancers, one study for cutaneous T-cell lymphoma showed that miR-122 expression was up-regulated in advanced stage mycosis fungoides (MF) and further induced during chemotherapy-induced apoptosis by regulating p53/Akt signaling (Manfè et al., 2012). Base on these studies, we checked the PI3K/Akt signaling to study the mechanisms of miR-122 on biological effect in RCC cells. Our data showed that overexpression of miR-122 affected the phosphorylation status of Akt but not the amount of Akt protein at the protein levels. We also observed that miR-122 activated mTOR and its downstream targets, p70S6K and 4E-BP1 in A498 and 786-O cells. These results indicate that miR-122 functions as an oncogenic miRNA in RCC, promoting tumor cell growth through activating PI3K/Akt signaling. Our findings are consistent with a previous study by Manfè et al showing that miR-122 regulated PI3K/Akt signaling in human cutaneous T-cell lymphoma. Next, we will analyze the roles and mechanisms of miR-122 in tumorgenesis and progression of renal cell carcinoma in vivo.

In conclusion, for the first time, our study demonstrates that miR-122 promotes renal cell carcinoma proliferation and increases the ability of invasion and migration through activating PI3K/Akt signaling pathway. Our findings suggest that miR-122 might be a promising target for the treatment of renal cell carcinoma in future.

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References


MicroRNA-122 Functional Roles in Renal Cell Carcinoma Cells

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