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ORIGINAL ARTICLE

MicroRNA-22 regulates the proliferation, drug sensitivity and metastasis of human glioma cells by targeting SNAIL1

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Summary

Purpose: *Gliomas are aggressive brain tumors accounting for significant mortality across the globe. Biomarkers for early detection and therapeutic targets for efficient treatment are lacking for glioma. This study was undertaken to investigate the role and therapeutic implications of miR-22 in glioma.*

Methods: *U-87 glioma cell line was used in this study. qRT-PCR was employed for expression analysis. MTT assay was used for determination of cell viability. Lipofectamine 2000 was used for transfection. Flow cytometry was used for cell analysis. Wound healing assay and transwell assay were used for monitoring cell migration and invasion. Western blot analysis was used for estimation of protein expression.*

Results: *The miR-22 expression was found decreased in glioma cells. Overexpression of miR-22 resulted in arrest of the U-87 glioma cells at G2/M checkpoint of the cell cycle. The percentage of apoptotic U-87 cells in G2/M phase were 13.05% in negative control (NC) and 29.06% in miR-22 mimics transfected cells. The cell cycle arrest promoted by miR-22 overexpression was also associated with depletion of cyclin B1 expression in U-87 cells. Furthermore, miR-22 could also significantly increase the sensitivity of glioma U-87 cells to cisplatin. The TargetScan analysis and dual luciferase assay showed SNAIL1 to be the target of miR-22. The expression of SNAIL1 was also enhanced in all the glioma cells and miR-22 overexpression could cause suppression of the SNAIL1 expression in U-87 cells. Furthermore, SNAIL1 silencing could also cause decline in the viability of the U-87 cells. The wound healing assay showed that miR-5 overexpression caused decrease in the migration of U-87 cells, while the transwell assay showed decline in the invasion of miR-22 mimics transfected U-87 cells.*

Conclusion: *Taken together, miR-22 may exhibit therapeutic implications in glioma and may prove useful in glioma treatment.*

Key words: *glioma, microRNA, cell cycle arrest, SNAIL1, migration, invasion*

Introduction

Accounting for about 77% of all the primary tumors of the brain, glioma includes all tumors of glial origin [1]. Considered to be among the most destructive human cancers, gliomas cause tremendous human mortality world over [2]. Surgery followed by chemo- and radiotherapy is generally employed for the management of gliomas [3]. Despite improvements in treatment, the average survival still remains 16 months for grade 4 gliomas [4]. Therefore, there is need for the identification of biomarkers for early detection and exploration of novel therapeutic targets for efficient treatment of gliomas [5]. Lots of studies carried out have shown that microRNAs (miRs) may exhibit therapeutic implications in treating human diseases such as cancer [6]. miRs regulate the expression of target genes via post transcriptional regulation and are around 19-23 nucleotides in length [7]. miR-22 has

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been shown to control a diverse array of molecular processes [8]. This miR has been shown to inhibit the growth of lung cancer cells via modulation of ErbB3 expression [9]. In another study, miR-22 has been reported to cause inhibition of gastric cancer cell growth and metastasis by suppressing CD151 [10]. In osteosarcoma, miR-22 targets HMGB1 to inhibit the cell proliferation [11]. Similarly, miR-22 inhibits the growth of prostate and cervical cancer cells via modulation of ATP citrate lyase [12]. In colon cancer cells, miR-22 exhibits the potential to reverse the paclitaxel-induced chemoresistance [13]. However, there is no report on the role and therapeutic implicates of miR-22 in glioma. Therefore, this study was undertaken to ascertain the role and therapeutic implications of miR-22 in glioma.

Methods

Cell culture

The normal astrocytes and the glioma cell lines U-87, U-118 MG, M059K and Hs 683 were obtained from Type Culture Collection of Chinese Academy of Sciences, Shanghai, China. The cell lines were cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640; Gibco, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (FBS) and 0.2% penicillin and streptomycin (Invitrogen, Carlsbad California, United States). All cells were cultured in a 5% CO_2 incubation chamber at 37°C. Because U-87 cells exhibited the lowest expression of miR-22, this cell line was used for further studies.

Cell viability

To determine the proliferation rate of the U-87 cells, the cells were transfected and incubated for 24 h and then incubated with MTT (500 µg/mL) for another 4 h. About 500 µl dimethyl sulfoxide (10%) was then added to dissolve the blue formazan crystals formed. Finally, the optical density (OD) was taken at 570 nm at different time intervals (0, 12, 24, 48 and 96 h) and cell viability was evaluated as the percentage of the control.

Cell transfection

The miR-22 mimics and negative control (NC) were synthesized by RiboBio (Guangzhou, China). Transfection was then carried out by the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. As the U-87 cells reached 80% confluence, appropriate concentrations of miR-22 mimics or NC were transfected into these cells.

Flow cytometric cell cycle analysis

After transfection the U-87 cells were cultured for 24 h at 37°C. Then, the cells were harvested and washed with phosphate buffered saline (PBS). Afterwards, the U-87 cells were stained with propidium iodide (PI) and the distribution of the cells in cell cycle phases was assessed by FACS flow cytometer.

Dual-luciferase reporter assay

The miR-22target was identified by TargetScan online software (http://www.targetscan.org). The miR-22 mimics or NC were co-transfected with Plasmid pGL3-BCl-2´-UTR-WT or pGL3-BCl-2´-UTR-MUT into U-87 cells. Dual-luciferase reporter assay (Promega) was carried out at 48 h after transfection. *Renilla* luciferase was used for normalization.

Cell invasion assay

The effects of miR-22 overexpression on the invasion ability of U-87 cells was determined by transwell assay (8 mm pore size, Corning, NY, USA) with Matrigel (Millipore, Billerica, USA). The U-87 cells were transfected with miR-22 mimics and around 200 ml cell cultures were placed onto the upper chamber and only medium was added in the bottom chamber. After 24 h of incubation, the cells were removed from the upper chamber and the cells that invaded via the chambers were fixed with methyl alcohol and subsequently stained with crystal violet. Inverted microscope was used to count the number of invaded cells at 200× magnification.

Cell migration assay

After 24 h of miR-22 mimics and NC transfection of U-87 cells, RPMI 1649 medium was removed and cells were PBS-washed. A sterile pipette tip was employed to scratch a wound in each well and cells were washed again and a picture was taken. The plates were cultured for 24 h and a picture was taken again under an inverted microscope.

Western blotting

The transfected U-87 glioma cells were harvested and lysed with lysis buffer and the protein extracts were boiled for 10 min in the presence of loading buffer followed by separation of cell extracts using 15% SDS-PAGE gel. The samples were then put onto polyvinylidene fluoride membranes and blocked using 5% skimmed milk powder. Membrane incubation with primary antibodies was then performed overnight at 4°C. This was followed by incubation of the membranes with horseradish peroxidase-linked biotinylated secondary antibodies at 1:1,000 dilution for 2 h. Washing of the membranes with PBS was followed to visualise the immunoreactive bands using the ECL-PLUS/Kit according to the manufacturer's instructions. The immune complexes development was carried out using an ECL detection kit according to the manual protocol (ECL GST western blotting detection kit, Pierce Biotechnology, Inc., Waltham, MA, USA). The bands were analyzed using the GelGDoc2000 imaging system (Bio-Rad Laboratories GmbH, Munich, Germany).

Statistics

The experiments were carried out in triplicate and the values were shown as mean±SD. Student's *t*-test (for comparison between two samples) and one way ANOVA followed by Tukey's test (for comparison between more than two samples) were used for statistical analysis using GraphPad prism software 7. The values were considered significant at $p<0.05$.

Results

miR-22 is suppressed in glioma cells

The qRT-PCR was used for the determination of the expression of miR-22 in different glioma cells. The results revealed that miR-22 was significantly suppressed in the glioma cells as compared to the normal astrocytes (Figure 1A). The expression of miR-22 was found to be up to 9-fold higher in the glioma cells. The U-87 showed the highest expression (8.5-fold suppression) out of all the cell lines.

miR-22 inhibits the proliferation of U-87 glioma cells

To determine the effects of miR-22, the U-87 cells were transfected with miR-22 mimics. The expression of miR-22 in the transfected U-87 cells

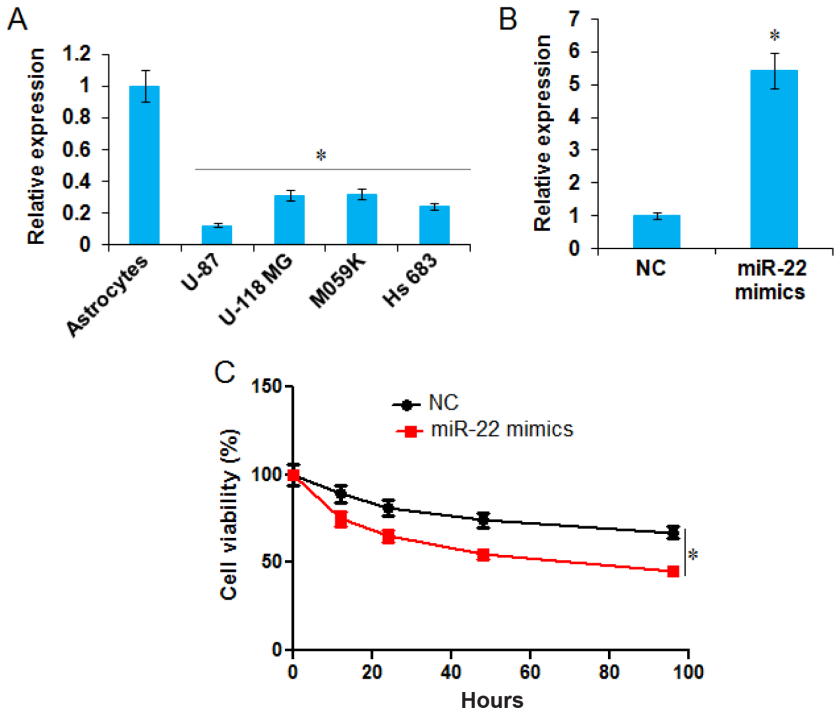

Figure 1. A: Expression of miR-22 in the normal astrocytes and four different glioma cells. **B:** expression of miR-22 in NC or miR-22 mimics transfected U-87 glioma cells. **C:** MTT assay showing the viability of U-87 cells transfected with NC or miR-22 mimics. The experiments were carried out in three biological replicates and expressed as mean ± SD (*p < 0.05).

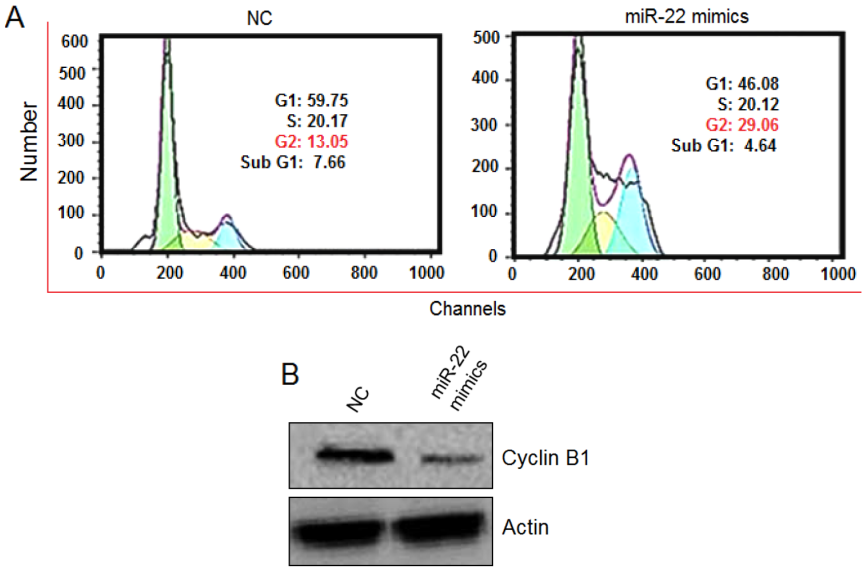

Figure 2. A: Cell cycle analysis of NC or miR-22 mimics transfected U-87 glioma cells showing induction of cell cycle arrest. **B:** Western blots showing the expression of cyclin B1 in NC or miR-22 mimics transfected U-87 glioma cells. The experiments were performed in triplicate.

was examined by qRT-PCR which showed 5.6-fold increases in the expression of miR-22 (Figure 1B). Afterwards, the cell viability was assessed at different time intervals (0, 12, 24, 48 and 96 h). What was found was that miR-22 overexpression caused significant decline in the viability of the U-87 cells (Figure 1C). After 96 h of incubation, the cell viability was 67% in the NC transfected cells as compared to 45% in miR-22 mimics transfected U-87 cells ($p<0.05$).

miR-22 induces G2/M arrest of U-87 glioma cells

To unveil the reasons underlying the inhibition of proliferation triggered by miR-22 overexpression, the miR-22 mimics-transfected U-87 cells were subjected to cell cycle analysis by flow cytometry. The results showed that miR-22 mimics transfected caused a remarkable increase in the G2/M phase of the cell cycle (Figure 2A). The percentage of G2/M phase cells was 13.05% in the NC

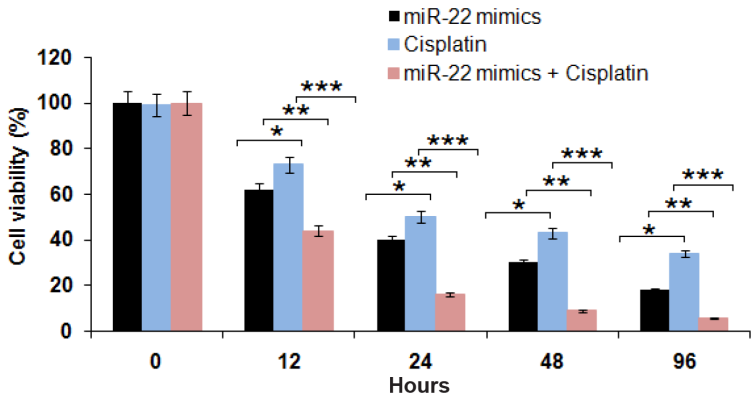

Figure 3. Effect of miR-22 overexpression on the cisplatin sensitivity of U-87 glioma cells. The Figure shows miR-22 overexpression enhances the sensitivity of U-87 glioma cells to the anticancer drug cisplatin. The experiments were performed in triplicate and expressed as mean ± SD (*$p<0.01$, **$p<0.001$ and ***$p<0.0001$).

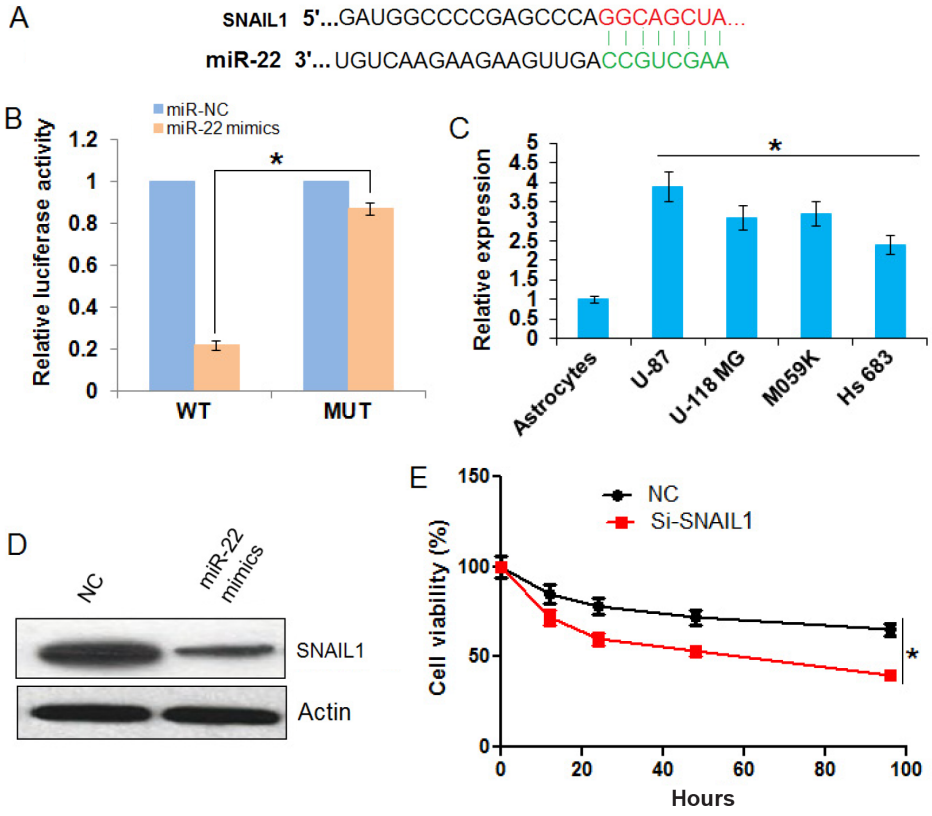

Figure 4. A: Identification of miR-22 target in glioma U-87 cells by TargetScan analysis. **B:** Dual luciferase assay. **C:** Expression of SNAIL1 in normal astrocytes and four different glioma cells. **D:** Western blots showing the expression of SNAIL1 in NC or miR-22 mimics transfected U-87 glioma cells. **E:** MTT assay showing the viability of the NC or miR-22 mimics transfected glioma cells. The experiments were carried out in triplicate and expressed as mean ± SD (*$p<0.05$).

transfected U-87 cells in comparison to 29.06% in miR-22 mimics transfected U-87 cells, suggestive of the G2/M arrest ($p<0.05$). Additionally, miR-22 overexpression also resulted in considerable decrease of the Cyclin B1 (Figure 2B).

miR-22 enhances the drug sensitivity of U-87 glioma cells

To assess whether miR-22 has any effect on the cisplatin sensitivity of the U-87 glioma cells, the cells were treated with 2 μM of miR-22 mimics or cisplatin plus miR-22 mimics transfection. The viability of these group of cells was monitored by MTT assay at 0, 12, 24, 48 and 96 h time intervals. What was found was that the cell viability of miR-22 mimics or cisplatin-treated cells was significantly higher as compared to U-87 cells treated with cisplatin plus transfected with miR-22 mimics (Figure 3). This suggests that miR-22 enhances the sensitivity of the glioma U-87 cells to cisplatin.

miR-22 targets SNAIL1 in U-87 glioma cells

The TargetScan analysis showed that miR-22 targets SNAIL1 in U-87 glioma cells (Figure 4A).

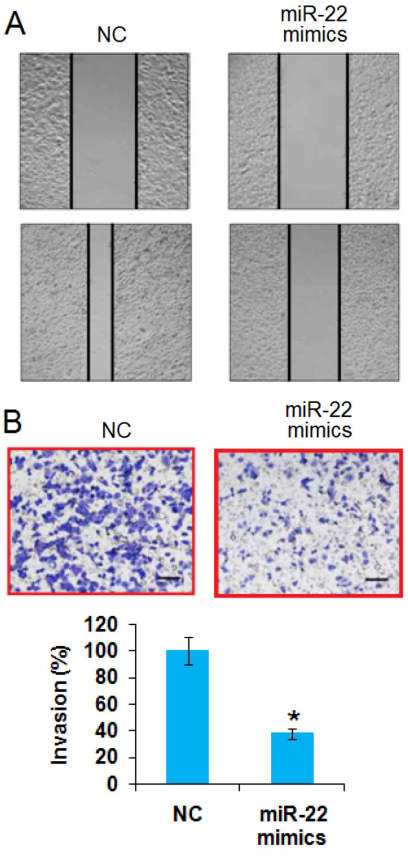

Figure 5. A: Wound-healing assay showing cell migration in NC or miR-22 mimics transfected U-87 cells. **B:** Transwell assay showing cell invasion in NC or miR-22 mimics transfected U-87 cells. The experiments were carried out in triplicate and expressed as mean ± SD (*$p<0.05$).

Dual luciferase also confirmed SNAIL1 as the target of miR-22 (Figure 4B). Therefore, the expression of SNAIL1 was also determined in all the glioma cells and the results showed that the expression of SNAIL1 was significantly increased in all the glioma cells (Figure 4C). The fold upregulation of SNAIL1 ranged between 2.4 to 3.9 in glioma cells. Nonetheless, miR-22 overexpression caused suppression of SNAIL1 in U-87 cells (Figure 4D). The effects of SNAIL1 silencing were also investigated on the U-87 cell proliferation and it was observed that silencing SNAIL1 in U-87 cells resulted in decrease of the U-87 cell proliferation (Figure 4E).

miR-22 inhibits the migration and invasion of U-87 cells

The effects of miR-22 on the migration of the U-87 cells were determined by wound healing assay. The results showed that miR-35 caused considerable reduction in the migration of the U-87 cells as evidenced from the scratch width (Figure 5A). The transwell assay also showed that miR-22 significantly suppressed the invasion of the U-87 cells. The migration of the U-87 cells was suppressed up to 62% upon miR-22 overexpression (Figure 5B).

Discussion

Being aggressive brain tumors, gliomas cause considerable mortality worldwide. The lack of reliable and efficient therapeutic targets/agents is one of the principal drawbacks in the treatment of glioma [14]. The diverse role that miRs play in humans by controlling the expression of approximately 30% of the human genes indicates that miRs may prove useful therapeutic targets for treating human diseases, including cancer [15]. Herein, we investigated the role of miR-22 in glioma. The findings from the qRT-PCR indicated that the expression of miR-22 was significantly suppressed in glioma cells. In epithelial ovarian cancer, miR-22 has also been shown to suppress cell proliferation and has been considered as biomarker for its prognosis [16]. However, overexpression of miR-22 by transfection of the glioma cells with miR-22 mimics resulted in suppression of the viability of U-87 cells. These findings are all in concordance with previous observations wherein miR-22 has been shown to regulate the growth of a diversity of cancer cells [17]. For instance, miR-22 has been shown inhibit the growth of gastric, osteosarcoma and bladder cancer cells [10-12]. The cell cycle analysis showed that miR-22 overexpression resulted in the arrest of the U-87 in the G2/M phase of the cell cycle which was concomitant with depletion of cyclin

B1 expression. These observations are also complemented by previous studies wherein miR-22 has been shown to have a regulatory role in cell cycle, for example, in colon and liver cancer cells miR-22 has been reported to cause significant inhibition of cyclin A expression [18]. miR-22 has also been shown to regulate the sensitivity of colon cancer cells to paclitaxel [13] and 5-fluorouracil sensitivity in colorectal cancer cells [19]. Herein, we examined the effect of miR-22 on the sensitivity of the U-87 glioma cells to cisplatin and the results showed that miR-22 enhanced the sensitivity of the U-87 to the anticancer drug cisplatin. Additionally, miR-22 also suppressed the migration and invasion of the U-87 glioma cells. These results are in agreement with previous studies [11,12] wherein miR-22 has been shown to suppress the migration and invasion of the ovarian and gastric cancer cells, suggestive of the anti-metastatic potential of miR-22.

Conclusion

The findings of the present study indicate that miR-22 suppresses the growth of the glioma cells by post-transcriptional suppression of SNAIL1. Additionally, miR-22 also induced cell cycle arrest and inhibited the migration and invasion of the glioma cells. Hence, miR-22 may exhibit therapeutic implications in glioma and may prove useful in glioma treatment.

Conflict of interests

The authors declare no conflict of interests.

References

1. Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathologica 2005;109:93-108.
2. Ostrom QT, Bauchet L, Davis FG et al. The epidemiology of glioma in adults: a "state of the science" review. Neurooncology 2014;16:896-913.
3. Malmer B, Iselius L, Holmberg E, Collins A, Henriksson R, Grönberg H. Genetic epidemiology of glioma. Br J Cancer 2001;84:429.
4. Bondy ML, Scheurer ME, Malmer B et al. Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. Cancer 2008;113:1953-68.
5. Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. Nat Rev Neurol 2006;2:494.
6. Garzon R, Calin GA, Croce CM. MicroRNAs in cancer. Annu Rev Medicine 2009;60:167-79.
7. Aqeilan RI, Calin GA, Croce CM. miR-15a and miR-16-1 in cancer: discovery, function and future perspectives. Cell Death Differentiation 2010;17:215.
8. Xu D, Takeshita F, Hino Y et al. miR-22 represses cancer progression by inducing cellular senescence. J Cell Biol 2011;193:409-24.
9. Ling B, Wang GX, Long G, Qiu JH, Hu ZL. Tumor suppressor miR-22 suppresses lung cancer cell progression through post-transcriptional regulation of ErbB3. J Cancer Res Clin Oncol 2012;138:1355-61.
10. Wang X, Yu H, Lu X, Zhang P, Wang M, Hu Y. MiR-22 suppresses the proliferation and invasion of gastric cancer cells by inhibiting CD151. Bioch Biophys Res Commun 2014;445:175-9.
11. Guo S, Bai R, Liu W et al. miR-22 inhibits osteosarcoma cell proliferation and migration by targeting HMGB1 and inhibiting HMGB1-mediated autophagy. Tumor Biol 2014;35:7025-34.
12. Xin M, Qiao Z, Li J et al. miR-22 inhibits tumor growth and metastasis by targeting ATP citrate lyase: evidence in osteosarcoma, prostate cancer, cervical cancer and lung cancer. Oncotarget 2016;7:44252.
13. Li J, Zhang Y, Zhao J, Kong F, Chen Y. Overexpression of miR-22 reverses paclitaxel-induced chemoresistance through activation of PTEN signaling in p53-mutated colon cancer cells. Molec Cell Biochem 2011;357:31-8.
14. Peckham-Gregory EC, Montenegro RE, Stevenson DA et al. Evaluation of racial disparities in pediatric optic pathway glioma incidence: Results from the Surveillance, Epidemiology, and End Results Program, 2000-2014. Cancer Epidemiol 2018;54:90-4.
15. Iorio MV, Croce CM. MicroRNAs in cancer: small molecules with a huge impact. J Clin Oncol 2009;27:5848.
16. Wan WN, Zhang YQ, Wang XM et al. Down-regulated miR-22 as predictive biomarker for prognosis of epithelial ovarian cancer. Diagn Pathol 2014;9:178.
17. Song SJ, Pandolfi PP. miR-22 in tumorigenesis. Cell Cycle 2014;13:11-2.
18. Yang F, Hu Y, Liu HX, Wan YJ. MiR-22-silenced cyclin A expression in colon and liver cancer cells is regulated by bile acid receptor. J Biol Chem 2015;290:6507-15.
19. Zhang H, Tang J, Li C et al. MiR-22 regulates 5-FU sensitivity by inhibiting autophagy and promoting apoptosis in colorectal cancer cells. Cancer Lett 2015;356:781-90.
20. Li J, Liang S, Yu H, Zhang J, Ma D, Lu X. An inhibitory effect of miR-22 on cell migration and invasion in ovarian cancer. Gynecol Oncol 2010;119:543-8.
21. Guo MM, Hu LH, Wang YQ et al. miR-22 is down-regulated in gastric cancer, and its overexpression inhibits cell migration and invasion via targeting transcription factor Sp1. Med Oncol 2013;30:542.