Genetics and Molecular Research

Link to retraction noticed

Decreased microRNA-198 expression and its prognostic significance in human glioma

H.B. Man[1,2], W.P. Bi[3] and H.H. Man[4]

[1]Department of Emergency, Wendeng Central Hospital of Weihai, Weihai, Shandong, China
[2]Department of Neurosurgery, Wendeng Central Hospital of Weihai, Weihai, Shandong, China
[3]Department of Gastroenterology, Wendeng Central Hospital of Weihai, Weihai, Shandong, China
[4]Department of Neurology, Wendeng Central Hospital of Weihai, Weihai, Shandong, China

Corresponding author: H.H. Man
E-mail: doc...hnh@163.com

Genet. Mol. Res. 15 (2): gmr15027656
Received September 16, 2015
Accepted December 10, 2015
Published May 25, 2016
DOI: http://dx.doi.org/10.4238/gmr.15027656

ABSTRACT. Previous evidence has shown the association of aberrant miR-198 expression with tumorigenesis and progression of many human malignancies. However, its involvement in human glioma is still unclear. Therefore, the aim of the current study was to investigate the expression and function of miR-198 in human gliomas. Using real-time quantitative RT-PCR, we examined miR-198 expression in 122 pairs of human gliomas and matched non-neoplastic brain tissues. The association of miR-198 expression with clinicopathological factors was also analyzed. Then, the effects of miR-198 on the biological behavior of glioma cells *in vitro* were evaluated. Our results showed that miR-198 expression was significantly downregulated in gliomas compared with corresponding non-neoplastic brain tissues ($P < 0.001$). Furthermore, low levels of miR-198 were associated with a higher WHO grade and lower Karnofsky performance status (KPS) score. A multivariate

Cox regression analysis identified decreased miR-198 expression as an independent factor predicting poor prognosis for glioma patients. Lastly, *in vitro* functional analysis revealed that overexpression of miR-198 in U87 cells reduced cell proliferation, promoted cell apoptosis, and inhibited cell invasion and migration. Taken together, these findings indicate that miR-198 may act as a tumor suppressor in human glioma, and may serve as a novel target for molecular therapies of this disease.

Key words: MicroRNA-198; Glioma; Real-time quantitative RT-PCR; Prognosis

INTRODUCTION

Gliomas, arising from glial cells, are the most common and aggressive primary tumor type of the central nervous system (CNS) (Furnari, et al., 2007). In spite of significant improvements in therapeutic technologies, such as those in neurosurgery, radiotherapy, chemotherapy, and photodynamic therapy, the median survival time of high-grade glioma patients has remained at 12-15 months over the past decade, and the cumulative 5-year survival rate remains lower than 30% (Jansen et al., 2004; Wen and Kesari, 2008). The poor prognosis of gliomas is largely attributed to their rapid growth, invasive/migratory nature, and high rate of recurrence. Previous studies have revealed many genes and signaling pathways that are dysregulated in gliomas (Zhou et al., 2013; Li and Liu, 2015; Liao et al., 2015), but the highly complex molecular mechanisms underlying glioma tumorigenesis and progression are still obscure. Therefore, it is necessary to identify key candidate molecules for its early diagnosis, effective therapy, and prognostic evaluation.

MicroRNAs (miRNAs) are a class of naturally occurring, short (approximately 22 nucleotides in length), single-stranded non-protein-coding RNAs that negatively regulate gene expression (Bartel, 2009). It is estimated that as many as 60% of genes are regulated by miRNAs (Esquela-Kerscher and Slack, 2006). Functionally, miRNAs suppress translation or promote the degradation of target messenger RNAs (mRNAs) through base pairing with the 3'-untranslated regions of the target mRNAs (Bartel, 2004, 2009). Previous research has shown that miRNAs have critical roles in various biological processes, such as development, differentiation, cell growth, inflammation, stress response, and endocrine homeostasis (Cech and Steitz, 2014). Emerging evidence demonstrates that aberrant miRNA expression is highly associated with cancer initiation and progression, which may provide new promising targets to treat cancer (Zhang et al., 2007; Dieckmann et al., 2012; Takahashi et al., 2012). miRNAs can function as either oncogenes or tumor suppressor genes depending on the roles of their target genes. Dysregulation or dysfunction of miRNAs is involved in many processes of tumor progression, including cell proliferation, apoptosis, invasion, metastasis, angiogenesis, and epithelial-to-mesenchymal transition (Shi et al., 2015; Wu et al., 2014; Yin et al., 2015). Therefore, miRNAs may be useful for cancer diagnosis and prognosis, and may act as potential novel therapeutic targets.

miR-198 is a recently identified cancer-related miRNA. Specifically, miR-198 has been observed to be downregulated in lung cancer (Yang et al., 2014), colorectal cancer (Wang et al., 2014), hepatocellular carcinoma (Tan et al., 2011), pancreatic cancer (Marin-Muller et al., 2013), and prostate cancer (Ye et al., 2013), and acts as a potential tumor suppressor in

these cancers. Interestingly, miR-198 was also reported to be upregulated in retinoblastoma and squamous cell carcinoma of the tongue (Wong et al., 2008; Zhao et al., 2009), indicating that miR-198 may not behave as a tumor suppressor in all cancers. However, the expression of miR-198 and its significance in human glioma has not yet been evaluated. In the present study, we examined miR-198 expression in glioma specimens and cell lines. The relationship between miR-198 expression and clinicopathological features as well as patient survival was then analyzed. Furthermore, we evaluated the biological roles of miR-198 in glioma cells.

MATERIAL AND METHODS

Patients and tissue samples

This study was approved by the Research Ethics Committee of Wendeng Center Hospital of Weihai, China. Written informed consent was obtained from all study participants. All specimens were handled and made anonymous according to ethical and legal standards.

In total, 122 pairs of glioma and adjacent non-neoplastic brain tissues were surgically collected from the Department of Neurosurgery at Wendeng Center Hospital of Weihai, China, from 2006 to 2010. All patients underwent tumor resection and the diagnosis of glioma were confirmed by histological examination. According to the WHO classification, 33 patients were classified as low-grade, including 13 pilocytic astrocytomas (WHO I) and 20 diffuse astrocytomas (WHO II); 89 were classified as high-grade, including 38 anaplastic astrocytomas (WHO III); and 51 primary glioblastomas (WHO IV). None of the patients had undergone chemotherapy or radiotherapy before surgery. All tissue samples were flash-frozen and stored at -80°C until RNA extraction. Patient characteristics are summarized in Table 1. Follow-up data were available for all patients. Overall survival was defined as the time from diagnosis to patient death, or for living patients, to the date of last follow-up.

Cell lines and miRNA transfection

Primary normal human astrocytes (NHA) were purchased from the ScienCell Research Laboratories (Carlsbad, CA, USA), and cultured under conditions as instructed by the manufacturer. Glioma cell lines H4, LN229, U87, and U251 were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China), and grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin sulfate. Cultures were incubated in a humidified atmosphere of 5% CO_2 at 37°C.

miR-198 mimics and negative control miRNA (miR-NC) were designed and synthesized by GenePharma Co. (Shanghai, China). Glioma cells were plated onto a six-well plate at a density of 3 x 10^5 cells/well for approximately 24 h prior to transfection. Transient transfections of miR-198 mimics or miR-NC (20 nM) were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer instructions. After 24 h, the cells were collected for further analysis.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from clinical specimens using TRIzol reagent (Invitrogen)

according to manufacturer instructions. RNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Complementary DNA (cDNA) was synthesized from isolated RNA using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed with a TaqMan MicroRNA Assay Kit (Applied Biosystems) on an ABI7500 Real-Time PCR Detection System (Applied Biosystems). Quantitative PCR was conducted at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. U6 small nuclear RNA was used as an internal control. All reactions were run in triplicate. The cycle threshold (Ct) values were recorded, and the relative amount of miR-198 to U6 was calculated using the equation $2^{-\Delta Ct}$, where $\Delta Ct = (Ct_{miR-198} - Ct_{U6})$.

Cell proliferation assay

Cell proliferation was analyzed using the MTT assay. Briefly, approximately 1 x 10^3 cells were seeded onto a 96-well plate and incubated for 1, 2, 3, and 4 days. At the indicated time points, 20 µL MTT (5 mg/mL) (Sigma, St. Louis, MO, USA) were added to each well and incubated for another 4 h. Then, the supernatants were removed and 150 µL DMSO (Sigma) was added to terminate the reaction. The absorbance value (OD) was measured at 490 nm on a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Detection of apoptosis by flow cytometry

Forty-eight hours after transfection, the glioma cells were harvested, washed, and resuspended in ice-cold PBS. The cells were then incubated with propidium iodide (10 mg/mL; Sigma) and Annexin V-FITC (5 µg/mL; BD Biosciences, San Jose, CA, USA) in the dark for 15 min at room temperature and examined by flow cytometry (FACScan; BD Biosciences).

Cell invasion and migration assays

Six-well transwell chambers (8-mm pore size; Corning, New York, NY, USA) were used to investigate cell invasion and migration. For the migration assay, approximately 1 x 10^5 glioma cells in serum-free media were seeded into the upper chambers after miR-198 mimic or miR-NC transfection. The lower chambers contained media with 20% FBS to stimulate migration. Following a 48 h-incubation, the cells located on the surface of the lower chamber were stained and counted using a microscope (Olympus Corp., Tokyo, Japan). For the invasion assay, the upper chambers were first covered with 5 mg/mL Matrigel, and the other steps were the same as for the migration assay.

Statistical analysis

All statistical analyses were performed using the SPSS 16.0 software package (SPSS, Chicago, IL, USA). Significant differences between groups were estimated by the Student *t*-test and one-way analysis of variance (ANOVA). Survival curves were constructed with the Kaplan-Meier method and compared by log-rank test. Differences in survival variables were evaluated using a multivariate Cox proportional hazard regression analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Downregulation of miR-198 in glioma tissues and cell lines

miR-198 expression was detected in glioma tissues and cell lines and normalized to U6 small nuclear RNA. As shown in Figure 1A, we found that miR-198 expression was significantly lower in glioma tissues than in non-neoplastic brain tissues ($P < 0.001$). Additionally, there was a significant difference in miR-198 expression between high-grade (WHO grades III-IV) and low-grade (WHO grades I-II) glioma specimens ($P < 0.001$). The miR-198 expression in the four glioma cell lines was also clearly downregulated (Figure 1B). The U87 cell line, which exhibited the lowest miR-198 expression among all the tested glioma cell lines, was selected for miR-198 mimic transfection and further studies.

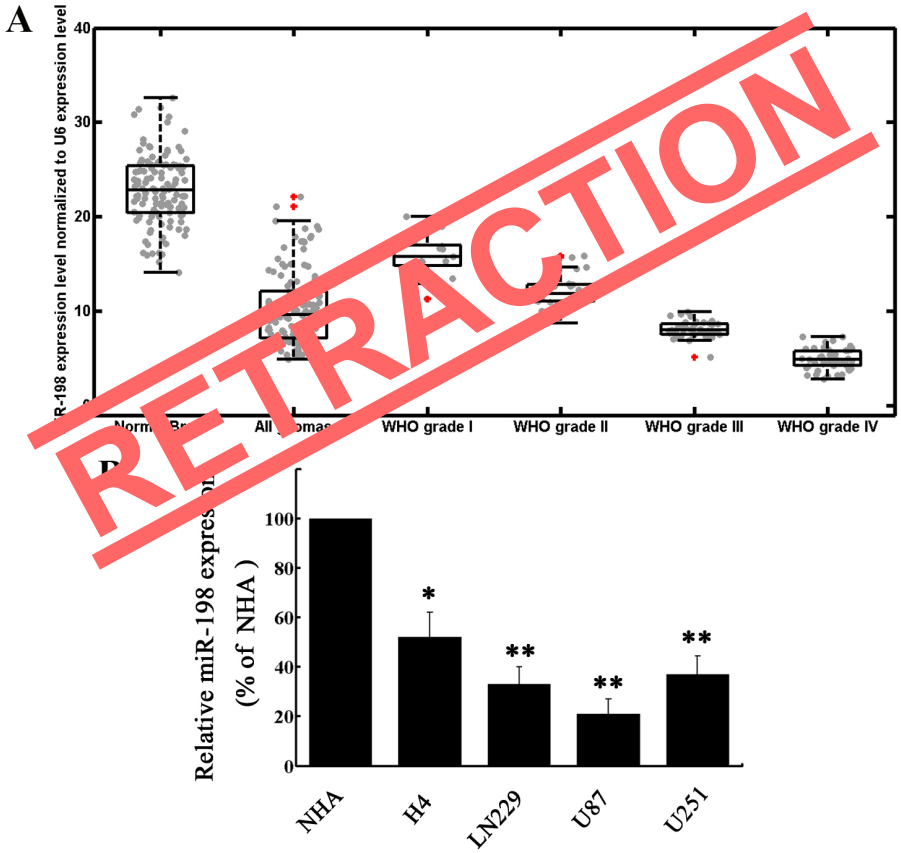

Figure 1. Relative expression levels of miR-198 in glioma tissues and cell lines. **A.** miR-198 expression was signiðcantly lower in glioma tissues than in the corresponding non-neoplastic brain tissues. Additionally, there was a significant difference in miR-198 expression between high-grade (III-IV) and low-grade (I-II) glioma specimens ($P < 0.01$). miR-198 expression levels were calculated by the $2^{-\Delta Ct}$ method and normalized to U6 small nuclear RNA. **B.** miR-198 expression was down-regulated in the glioma cell lines H4, LN229, U87, and U251, compared to primary normal human astrocytes (NHA). *$P < 0.05$; **$P < 0.01$.

Association of miR-198 expression with clinicopathological parameters

Next, the clinicopathological significance of miR-198 expression in human glioma was analyzed. We divided the patients into the following two groups according to their miR-198 expression levels using the median value as the cutoff: high miR-198 expression group (N = 61) and low miR-198 expression group (N = 61). As shown in Table 1, miR-198 downregulation was significantly associated with high WHO grade (P = 0.005) and low Karnofsky performance status (KPS) score (P < 0.001).

Table 1. Association of miR-198 expression with different clinicopathological features of human gliomas.

Clinicopathological features	No. of cases	miR-198 expression		P value
		High N (%)	Low N (%)	
Age (years)				
<55	67	35 (52.2%)	32 (47.8%)	0.398
≥55	55	26 (47.3%)	29 (52.7%)	
Gender				
Male	70	37 (52.9%)	33 (47.1%)	0.293
Female	52	24 (46.2%)	28 (53.8%)	
WHO grade				
I	13	11 (84.6%)	2 (15.4%)	
II	20	14 (70.0%)	6 (30.0%)	0.005
III	38	16 (42.1%)	22 (57.9%)	
IV	51	20 (39.2%)	31 (60.8%)	
KPS score				
<80	71	25 (35.2%)	46 (64.8%)	<0.001
≥80	51	36 (70.6%)	15 (29.4%)	

Prognostic value of miR-198 expression in glioma patients

We further evaluated whether miR-198 expression had a prognostic value for overall survival of the glioma patients. The Kaplan-Meier survival curves are shown in Figure 2. The survival rate of patients with low miR-198 expression was significantly lower than that of patients with high miR-198 expression (P < 0.001). Furthermore, the survival benefits were also found in patients with low WHO grade (P = 0.003) and high KPS score (P = 0.022). In the multivariate analysis, the Cox proportional hazard model involving miR-198 expression and various clinicopathological parameters identified low miR-198 expression (P = 0.005, RR = 8.86) and high WHO grade (P = 0.018, RR = 3.65) as independent factors predicting poor prognosis (Table 2).

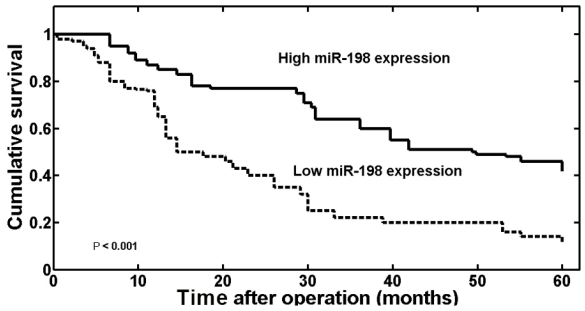

Figure 2. Kaplan-Meier survival curves of glioma patients based on miR-198 expression status. Patients in the low expression group had significantly poorer prognosis than those in the high expression group (P < 0.001, log-rank test).

Table 2. Univariate and multivariate analysis of overall survival in 122 patients with glioma.

Variables	Univariate log-rank test (P)	Cox multivariable analysis (P)	Relative risk (RR)
Age at diagnosis (years)			
<55 *vs* ≥55	0.38	-	-
Gender			
Male *vs* female	0.47	-	-
WHO grade			
I-II *vs* III-IV	0.003	0.018	3.65
KPS score			
<80 *vs* ≥80	0.022	0.07	1.19
MiR-198 expression			
High *vs* low	<0.001	0.005	8.86

Effects of miR-198 on the biological behavior of U87 cells

Lastly, we assessed the biological role of miR-198 in U87 cells. As shown in Figure 3A, the expression level of miR-198 in miR-198 mimic-transfected cells was signiðcantly higher than that in miR-NC-transfected cells (P < 0.01). The MTT assay showed that cell proliferation was significantly impaired after miR-198 mimic transfection (Figure 3B). We also observed that cell apoptosis was increased in miR-198 mimic-transfected cells (Figure 3C). Furthermore, transwell invasion/migration assays revealed an inhibitory effect of miR-198 on U87 cell invasion and migration (Figure 3D and E).

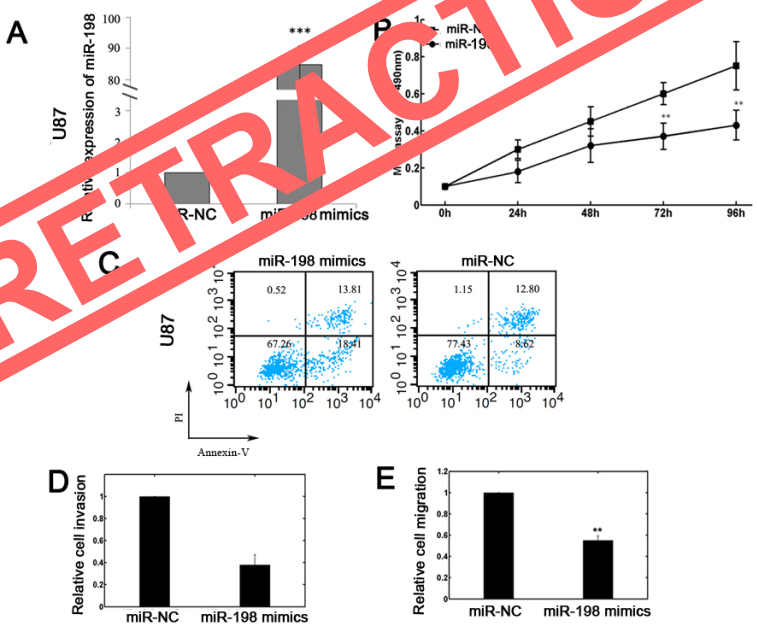

Figure 3. Effects of miR-198 on the biological behaviors of U87 cells. **A.** The expression level of miR-198 in miR-198 mimic-transfected cells was signiðcantly higher than that in negative control (NC)-transfected cells. ***P < 0.001. **B.** Cell proliferation was measured by MTT assays in U87 cells transfected with miR-198 mimic or NC. **P < 0.01. **C.** Apoptosis of U87 cells was detected by flow cytometric analysis after transfection with miR-198 mimic or NC. **D.** and **E.** miR-198 suppressed U87 cell invasion and migration *in vitro*. The transwell invasion and migration assays showed that the number of invaded or migrated cells was significantly lower in the miR-198-transfected group than in the NC-transfected group. **P < 0.01.

DISCUSSION

Identifying novel molecules that are associated with glioma development and progression may be helpful for improving the diagnosis, prevention, and treatment of this disease. The relationship between miRs and tumorigenesis has thus become a focus of current cancer research. Abnormal expression of several miRs have been reported in glioma. For example, Dontula et al. (2013) revealed that decreased miR-203 expression in glioma cells was associated with cell migration and invasion. Yu et al. (2012) found that overexpression of miR-34a *in vitro* suppressed the proliferation and induced cell apoptosis of U87 glioma cells. Moreover, low miR-326 expression in glioma was significantly associated with advanced pathological grade and low KPS score *in vivo* (Wang et al., 2013). Additionally, miR-203 downregulation and miR-372 upregulation were reported as unfavorable prognostic factors in patients with gliomas (He et al., 2013; Li et al., 2013). Together, these previous findings suggest that miRNAs play important roles in glioma initiation and development, and thus have a great potential as clinical targets.

In the present study, we observed low miR-198 expression in glioma specimens and cell lines, providing the first evidence that miR-198 downregulation is closely associated with glioma formation. Next, we investigated correlations between decreased miR-198 levels and clinicopathological features. We found that overexpression of miR-198 in U87 cells reduced cell proliferation, enhanced cell apoptosis, and impaired cell invasion and migration. These findings revealed that miR-198 may be involved in glioma progression, and thus that miR-198 may be investigated in future molecular-targeted therapies. Additionally, our results showed that glioma patients with low miR-198 levels tended to have shorter overall survival than that of patients with high miR-198 levels. Furthermore, the multivariate Cox hazard regression analysis identified low miR-198 expression as an independent indicator of unfavorable prognosis. To our knowledge, this is the first report on the expression and clinical significance of miR-198 in glioma.

The results herein regarding glioma are consistent with previous findings for other cancers. For example, decreased miR-198 expression in colorectal cancer was significantly associated with histological grade, local invasion, lymph node metastasis, and AJCC stage (Wang et al., 2014). Conversely, the overexpression of miR-198 in colorectal cancer cell lines *in vitro* inhibited cell proliferation, invasion, and migration by targeting fucosyl transferase 8 (FUT8). *In vivo*, restoration of miR-198 significantly inhibited colorectal cancer xenograft growth and invasion in nude mice. In pancreatic cancer, restoration of miR-198 resulted in reduced tumor growth and metastasis through direct targeting of MSLN, PBX-1, and VCP (Marin-Muller et al., 2013). Additionally, low miR-198 levels in pancreatic cancer tissue samples predicted shorter overall survival. Furthermore, Yang et al. (2014) reported that miR-198 inhibited proliferation and induced apoptosis of A549 lung cancer cells via FGFR1 targeting. Tan et al. (2011) found that miR-198 inhibited hepatocellular carcinoma cell invasion and migration by targeting the HGF/c-MET pathway. Taken together, these results indicate that miR-198 may serve as a tumor-suppressor in several types of human malignancies. However, the complex molecular mechanisms underlying low miR-198 expression in human malignancies and its function remain unknown. Therefore, future studies should be performed to elucidate the precise mechanisms by which miR-198 influences tumor formation and progression.

In conclusion, our research demonstrated that there was decreased miR-198 expression in glioma tissues and cell lines. We also showed that low miR-198 levels were correlated with

tumor progression and poor prognosis. Moreover, miR-198 expression was shown to influence biological behaviors of glioma cells *in vitro*. These findings suggest that miR-198 may act as a tumor suppressor in glioma initiation and development, and may also be a novel prognostic marker and a potential therapeutic target for this disease.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297. http://dx.doi.org/10.1016/S0092-8674(04)00045-5

Bartel DP (2009). MicroRNAs: target recognition and regulatory functions. *Cell* 136: 215-233. http://dx.doi.org/10.1016/j.cell.2009.01.002

Cech TR and Steitz JA (2014). The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* 157: 77-94. http://dx.doi.org/10.1016/j.cell.2014.03.008

Dieckmann KP, Spiekermann M, Balks T, Flor I, et al. (2012). MicroRNAs miR-371-3 in s[illegible]m as diagnostic tools in the management of testicular germ cell tumours. *Br. J. Cancer* 107: 1754-1760. http[illegible]ax.doi.org/[illegible]0.1038/bjc.2012.469

Dontula R, Dinasarapu A, Chetty C, Pannuru P, et al. (2013). MicroRNA 203 Mo[illegible]lates Glio[illegible]ll [illegible]igration via Robo1/ERK/MMP-9 Signaling. *Genes Cancer* 4: 285-296. http://dx.doi.org/[illegible]

Esteller M (2011). Non-coding RNAs in human disease. *Nat. Rev. G[illegible]et.* 12: 61[illegible]74. http[illegible]/dx.doi.o[illegible]10.1038/nrg3074

Furnari FB, Fenton T, Bachoo RM, Mukasa A, et al. (2007). M[illegible]gna[illegible]strocy[illegible]c g[illegible]oma genetic, biology, and paths to treatment. *Genes Dev.* 21: 2683-2710. http://dx.doi[illegible]g/10.1101/ga[illegible]5967[illegible]

He J, Deng Y, Yang G and Xie W (2013). MicroR[illegible]-203 [illegible]own-regulati[illegible]n is asso[illegible]ed with unfavorable prognosis in human glioma. *J. Surg. Oncol.* 108: 121[illegible]5. http://dx[illegible]oi.org/[illegible]0.1002/js[illegible]5315

Jansen M, de Witt Hamer PC, Witmer [illegible], Troost [illegible], et al. [illegible]004. Cur[illegible]t perspectives on antiangiogenesis strategies in the treatment of malig[illegible]t gli[illegible]. *Br[illegible] Brain[illegible]s. Rev.* 45: 143-163. http://dx.doi.org/10.1016/j.brainresrev.2004.03.00[illegible]

Li G, Zhang Z, Tu Y, Ji[illegible], et al. [illegible]013). C[illegible]rela[illegible]of mi[illegible]RNA-372 upregulation with poor prognosis in human glioma. *Diagn. Path[illegible]* 8: 1[illegible]ttp://dx[illegible]oi.org/[illegible]0.1186/[illegible]46-1596-8-1

Li L and Li[illegible] (2015). Aging-relate[illegible]gene si[illegible]ature regulated by Nlrp3 predicts glioma progression. *Am. J. Cancer Res.* 5: [illegible]42-[illegible]

Liao CK, Fa[illegible] K[illegible], Cha[illegible]wu C[illegible], et al. (2015). Depletion of B cell CLL/Lymphoma 11B Gene Expression Represses Glioma[illegible]ll Gr[illegible]th. *[illegible]. Neurobiol.*

Marin-Muller C, Li D, Bharadwaj U, Li M, et al. (2013). A tumorigenic factor interactome connected through tumor suppressor microRNA-198 in human pancreatic cancer. *Clin. Cancer Res.* 19: 5901-5913. http://dx.doi.org/10.1158/1078-0432.CCR-12-3776

Shi H, Ji Y, Zhang D, Liu Y, et al. (2015). MiR-135a inhibits migration and invasion and regulates EMT-related marker genes by targeting KLF8 in lung cancer cells. *Biochem. Biophys. Res. Commun.* 465: 125-130. http://dx.doi.org/10.1016/j.bbrc.2015.07.145

Takahashi M, Cuatrecasas M, Balaguer F, Hur K, et al. (2012). The clinical significance of MiR-148a as a predictive biomarker in patients with advanced colorectal cancer. *PLoS One* 7: e46684. http://dx.doi.org/10.1371/journal.pone.0046684

Tan S, Li R, Ding K, Lobie PE, et al. (2011). miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. *FEBS Lett.* 585: 2229-2234. http://dx.doi.org/10.1016/j.febslet.2011.05.042

Wang M, Wang J, Kong X, Chen H, et al. (2014). MiR-198 represses tumor growth and metastasis in colorectal cancer by targeting fucosyl transferase 8. *Sci. Rep.* 4: 6145. http://dx.doi.org/10.1038/srep06145

Wang S, Lu S, Geng S, Ma S, et al. (2013). Expression and clinical significance of microRNA-326 in human glioma miR-326 expression in glioma. *Med. Oncol.* 30: 373. http://dx.doi.org/10.1007/s12032-012-0373-y

Wen PY and Kesari S (2008). Malignant gliomas in adults. *N. Engl. J. Med.* 359: 492-507. http://dx.doi.org/10.1056/NEJMra0708126

Wong TS, Liu XB, Wong BY, Ng RW, et al. (2008). Mature miR-184 as Potential Oncogenic microRNA of Squamous

Cell Carcinoma of Tongue. *Clin. Cancer Res.* 14: 2588-2592. http://dx.doi.org/10.1158/1078-0432.CCR-07-0666

Wu YY, Chen YL, Jao YC, Hsieh IS, et al. (2014). miR-320 regulates tumor angiogenesis driven by vascular endothelial cells in oral cancer by silencing neuropilin 1. *Angiogenesis* 17: 247-260. http://dx.doi.org/10.1007/s10456-013-9394-1

Yang J, Zhao H, Xin Y and Fan L (2014). MicroRNA-198 inhibits proliferation and induces apoptosis of lung cancer cells via targeting FGFR1. *J. Cell. Biochem.* 115: 987-995. http://dx.doi.org/10.1002/jcb.24742

Ye L, Li S, Ye D, Yang D, et al. (2013). Livin expression may be regulated by miR-198 in human prostate cancer cell lines. *Eur. J. Cancer* 49: 734-740. http://dx.doi.org/10.1016/j.ejca.2012.08.029

Yin P, Peng R, Peng H, Yao L, et al. (2015). MiR-451 suppresses cell proliferation and metastasis in A549 lung cancer cells. *Mol. Biotechnol.* 57: 1-11. http://dx.doi.org/10.1007/s12033-014-9796-3

Yu X, Zhang W, Ning Q and Luo X (2012). MicroRNA-34a inhibits human brain glioma cell growth by down-regulation of Notch1. *Journal of Huazhong University of Science and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban* 32: 370-374.

Zhang B, Pan X, Cobb GP and Anderson TA (2007). microRNAs as oncogenes and tumor suppressors. *Dev. Biol.* 302: 1-12. http://dx.doi.org/10.1016/j.ydbio.2006.08.028

Zhao JJ, Yang J, Lin J, Yao N, et al. (2009). Identification of miRNAs associated with tumorigenesis of retinoblastoma by miRNA microarray analysis. *Childs Nerv. Syst.* 25: 13-20. http://dx.doi.org/10.1007/s00381-008-0701-x

Zhou J, Xu T, Yan Y, Qin R, et al. (2013). MicroRNA-326 functions as a tumor suppressor in glioma by targeting the Nin one binding protein (NOB1). *PLoS One* 8: e68469. http://dx.doi.org/10.1371/journal.po[illegible]0068469