miR-219-5p inhibits proliferation and clonogenicity in chordoma cells and is associated with tumor recurrence

WEI WEI, QIUHANG ZHANG, ZHENLIN WANG, BO YAN, YANJUN FENG and PU LI

Department of Otolaryngology, Head and Neck Surgery, Skull Base Surgery Center, Xuanwu Hospital, Capital Medical University, Beijing 100053, P.R. China

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Abstract. Chordoma is a rare malignant bone tumor that is usually localized to the skull base, vertebral column and sacrum. The transcription factor brachyury, which is encoded by the T gene, has a critical role in the development and progression of chordoma, although the mechanisms underlying brachyury regulation remain unclear. The aim of the current study was to identify and characterize microRNAs (miRs) that regulate brachyury expression in chordoma. MicroRNAs that target brachyury were predicted using miRanda and TargetScan. Using reverse transcription-quantitative polymerase chain reaction, miR-219-5p was shown to be significantly downregulated in chordoma tissues and the U-CH2 chordoma cell lines. A dual-luciferase reporter assay was used to validate the inhibitory effect of miR-219-5p on brachyury mRNA expression. The expression level of brachyury was downregulated in U-CH2 cells following transfection with miR-219-5p mimics and upregulated following transfection with the miR-219-5p inhibitor. The effects of miR-219-5p on the proliferation and clonogenicity of chordoma cells were assessed using cell counting kit-8, EdU and clone formation assays. These in vitro results indicated that miR-219-5p may have an important role in regulating the cell proliferation and clonogenicity of human chordoma cells, potentially by targeting brachyury. Furthermore, the associations between the expression levels of miR-219-5p and various clinicopathological factors were analyzed, and miR-219-5p expression was shown to correlate with tumor extent and recurrence. These results suggested that miR-219-5p functions as a tumor suppressor in chordoma and, therefore, that miR-219-50 may be a potential target for therapeutic intervention.

Introduction

Chordoma is a rare malignant bone tumor with an incidence of ~0.1/100,000 individuals (1). It is thought to originate from notochordal remnants and is usually localized to the skull base, vertebral column and sacrum. Despite improvements in therapeutic modalities, the treatment of chordoma remains challenging because of its location and local recurrence (2). Therefore, understanding the molecular mechanisms underlying the development and progression of chordoma is crucial to identify novel therapeutic targets.

Brachyury, which is a transcription factor encoded by the T gene, is specifically expressed in chordomas and in the notochord (3). T gene duplication is common in familial chordoma, while gain of the T locus has also been found in sporadic cases (4,5). Silencing of brachyury using short hairpin RNA has been shown to result in decreased cell proliferation and induction of a senescent phenotype (5,6). It is well-documented that brachyury orchestrates several downstream pathways in chordoma; however, the mechanisms underlying brachyury regulation remain unclear (7).

MicroRNAs (miRNAs or miRs) are a class of small, non-coding, single-stranded RNAs that can regulate the process of translation by binding to the 3’-untranslated region (UTR) of mature miRNAs. Importantly, miRNAs have been shown to be involved in tumorigenesis, angiogenesis, invasion and metastasis (8-10). Furthermore, miRNAs may served as oncogenes or tumor suppressors, and have therefore been reported to be dysregulated in various human cancers, including gastric cancer (11), colorectal cancer (12), thyroid cancer (13) and squamous cell carcinoma (14). However, a role for miRNAs in the regulation of brachyury expression in chordoma has yet to be elucidated.

In the present study, it was first demonstrated that miR-219-5p was downregulated in chordoma tissue samples and the U-CH2 cell line. Therefore, whether brachyury is a direct target of miR-219-5p was evaluated. In vitro analyses showed that miR-219-5p inhibited chordoma cell proliferation and clonogenicity by downregulating brachyury. The associations between miR-219-5p expression and various patient clinicopathological factors were further analyzed and it was
shown that miR-219-5p expression was correlated with the tumor extent and recurrence.

Materials and methods

Patients and tissue samples. Tissue samples were obtained from 40 patients who had undergone surgery at Xuanwu Hospital, Capital Medical University (Beijing, China) between February 2008 and February 2013. Freshly received tissues from the operating room were immediately frozen with liquid nitrogen. All patients had confirmed pathological diagnosis according to the World Health Organization classification (15). A total of 40 skull base chordoma samples and 10 non-neoplastic adjacent tissue samples (normal skeletal muscles) were used in accordance with the policies of the hospital's institutional review board. Written informed consent was obtained prior to initiation of the study from all patients. Medical records were reviewed to obtain clinical information for each chordoma case, including age, gender, tumor site, surgical approach and recurrence. The clinical findings of the 40 patients are listed in Table I.

Cell lines and reagents. The human chordoma cell line U-CH2 (ATCC, Manassas, VA, USA) was cultivated in Iscove's Modified Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and RPMI-1640 medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) at a ratio of 4:1 (vol/vol), supplemented with 10% fetal bovine serum (FBS; Seromed Biochrom, Berlin, Germany). Culture flasks were coated with rat tail type I collagen (BD Biosciences, San Diego, CA, USA) prior to use. The human embryonic kidney 293T, nasopharyngeal carcinoma CNE-2 and laryngeal carcinoma Hep-2 cell lines were cultivated at the Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences (Beijing, China). 293T cells were cultured in RPMI-1640 medium containing 10% FBS. CNE-2 and Hep-2 cell lines were cultured in Dulbecco's Modified Eagle's Medium containing 10% FBS. All cell lines were cultured in a humidified atmosphere at 37°C in 5% CO₂.

Bioinformatics analysis. miRNAs targeting the 3'-UTR of brachyury mRNA were predicted using miRanda (http://www.microrna.org/microrna/home.do) and TargetScan (http://www.targetscan.org).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from tissue samples and cell lines using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and was reverse transcribed using the TIANscript RT kit (Tiangen Biotech Co., Ltd., Beijing, China). The reverse transcription reaction was performed in a total volume of 14.5 µl, which included 2.5 µl (1 µg) total RNA, 2 µl dNTP mix (2.5 mM), 2 µl specific reverse transcription primer (10 µM) and 8 µl RNase-free water. The reaction mixture was initially incubated at 70°C for 5 min, followed by incubation on ice for 2 min. The tubes were centrifuged at 1,000 x g at 4°C for 5 sec, after which, 4 µl 5X First-Strand Buffer, 0.5 µl RNasin (40 U/µl) and 2 µl reverse transcriptase (200 U/µl) were added to a total reaction volume of 21 µl. The reaction mixture was then incubated at 42°C for 50 min, followed by incubation at 95°C for 5 min. qPCR was performed on an ABI PRISM 7500 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR FAST qPCR Master Mix (Kapa Biosystems, Inc., Wilmington, MA, USA), according to the manufacturer's protocol. The PCR conditions were as follows: 95°C for 3 min, 60°C for 20 sec; 40 cycles of 95°C for 3 sec, 60°C for 20 sec; and 95°C for 15 sec, 60°C for 15 sec and 95°C for 15 sec. Each sample was run in triplicate. The relative expression of miRNA compared with U6 was calculated using the 2^-ΔΔC_{t} method (16). The primer sequences, which were designed by Beijing Micoread Genetics, Co., Ltd. (Beijing, China), were as follows: miR-219-5p stem-loop, 5'-GTCGTA TCCAGTGCAAGGTCCAGTCCACTGATACCG AAGAATTGTC-3'; miR-219-5p forward, 5'-GGACGGTGG ATTGTCACAC-3'; common loop reverse, 5'-TCGTATCCA GTGCAAGGGTC-3'; U6 forward, 5'-CTCGTTCGCCAGCA CATATACT-3'; and U6 reverse, 5'-ACGGTCTCAGAATT GCGTGT-3'.
evaluated daily for 4 days using CCK-8 assays (Dojindo Molecular Technologies, Inc., Kumamoto, Japan), according to the manufacturer's protocol. Briefly, 10 µl CCK-8 was added to each well and, after 1 h, the optical density (OD) of the samples was measured at an absorbance of 450 nm using a microplate spectrophotometer (Epoch 2; BioTek Instruments, Winooski, VT, USA). Each transfection was performed in triplicate. The impact of miR-219-5p on U-CH2 cell proliferation was also assessed using the Cell-Light™ EdU detection kit (Guangzhou RiboBio, Co., Ltd.), according to the manufacturer's instructions. Briefly, at 72 h following transfection, cells were incubated with 10 µM EdU for 2 h prior to fixation with 4% paraformaldehyde for 30 min and permeabilization using 0.5% Triton X-100 for 10 min. Proliferating cells were

| N  | Gender | Age (years) | Tumor site            | Surgical approach | Initial/recurrent | miR  |
|----|--------|-------------|-----------------------|-------------------|------------------|------|
| 1  | F      | 25          | Clivus and CV         | TA                | Recurrent        | 0.451|
| 2  | M      | 25          | Clivus                | EEA               | Recurrent        | 0.656|
| 3  | F      | 41          | Clivus                | EEA               | Recurrent        | 0.600|
| 4  | F      | 13          | Clivus and CV         | TA and OCF        | Recurrent        | 0.196|
| 5  | M      | 32          | Clivus and SR         | TA                | Initial          | 0.960|
| 6  | M      | 62          | Clivus                | EEA               | Initial          | 2.646|
| 7  | F      | 39          | Clivus                | TA                | Recurrent        | 0.390|
| 8  | F      | 55          | Clivus                | EEA               | Initial          | 0.210|
| 9  | M      | 34          | Clivus                | EEA               | Initial          | 0.918|
| 10 | M      | 44          | Clivus                | EEA               | Initial          | 6.881|
| 11 | F      | 52          | Clivus                | EEA               | Initial          | 1.879|
| 12 | M      | 19          | Clivus                | EEA               | Initial          | 0.554|
| 13 | F      | 15          | Clivus                | TA                | Initial          | 0.230|
| 14 | M      | 50          | Petroclival region    | TA                | Recurrent        | 0.342|
| 15 | M      | 62          | Clivus                | EEA               | Initial          | 1.332|
| 16 | M      | 43          | Clivus and ASB        | EEA               | Recurrent        | 0.292|
| 17 | M      | 23          | Clivus                | EEA               | Initial          | 1.609|
| 18 | F      | 31          | Clivus                | TA                | Recurrent        | 2.154|
| 19 | M      | 42          | Petroclival region    | EEA               | Recurrent        | 0.662|
| 20 | M      | 33          | Clivus                | EEA               | Recurrent        | 0.380|
| 21 | F      | 49          | Clivus                | EEA               | Recurrent        | 0.898|
| 22 | F      | 32          | Clivus                | EOA               | Initial          | 1.822|
| 23 | M      | 56          | Clivus                | EEA               | Initial          | 1.589|
| 24 | M      | 51          | Clivus and SR         | EEA               | Recurrent        | 0.820|
| 25 | F      | 59          | Clivus                | EEA               | Initial          | 1.398|
| 26 | F      | 46          | Clivus and CV         | EOA               | Initial          | 0.853|
| 27 | M      | 69          | Clivus and CV         | EOA               | Initial          | 1.400|
| 28 | F      | 42          | Clivus and ASB        | EEA               | Recurrent        | 0.143|
| 29 | F      | 16          | Clivus                | EEA               | Initial          | 1.664|
| 30 | F      | 72          | Clivus and CV         | EOA               | Initial          | 0.882|
| 31 | F      | 60          | Clivus                | EEA               | Recurrent        | 1.526|
| 32 | F      | 66          | Clivus                | EEA               | Initial          | 0.151|
| 33 | M      | 43          | Clivus                | EEA               | Recurrent        | 1.058|
| 34 | M      | 22          | Clivus                | EEA               | Initial          | 4.216|
| 35 | M      | 32          | Clivus                | EEA               | Initial          | 1.211|
| 36 | M      | 42          | Clivus and CV         | EOA and OCF       | Initial          | 0.189|
| 37 | M      | 47          | Clivus and ASB        | EEA               | Recurrent        | 0.350|
| 38 | F      | 21          | Clivus                | EEA               | Initial          | 2.359|
| 39 | F      | 28          | Clivus                | EEA               | Recurrent        | 0.883|
| 40 | M      | 50          | Clivus                | EEA               | Initial          | 0.418|

CV, cervical vertebra; ASB, anterior skull base; SR, sellar region; TA, traditional approach; OCF, occipitocervical fusion; EEA, endoscopic endonasal approach; EOA, endoscopic transloral approach; miR, relative expression level of miR-219-5p.
stained with 100 μl 1X Apollo643 for 30 min, after which the cells were stained with 100 μl 1X Hoechst 33342. Fluorescent cells were counted using the automated Acumen eX3 Laser Scanning Cytometer (TTP Labtech, Ltd., London, UK).

Clone formation assay. U-CH2 or 293T cells were inoculated into rat tail type I collagen-coated 24-well plates at a density of 300 cells per well. After 18 h, the cells were transfected with 75 nM miR-219-5p mimics, NC, miR-219-5p inhibitor or NC inhibitor using Lipofectamine 2000, according to the manufacturer's protocol. Subsequently, the cells were cultured in a humidified chamber at 37°C containing 5% CO₂, with culture medium changed every 2 days. After 5 days of cultivation, the cells were stained with 20 μM Calcein-AM (Invitrogen; Thermo Fisher Scientific, Inc.) for 45 min. Cell clusters >200-μm in size were considered colonies and were counted using the automated Acumen eX3 Laser Scanning Cytometer.

Western blotting. At 72 h following transfection, U-CH2 cells were lysed in ice-cold radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). The protein concentration was estimated using a bicinchoninic acid protein assay (Beyotime Institute of Biotechnology). A total of 50 μg protein was separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat dry milk in 0.1% Tween-20 at 4°C for 1 h, then incubated with primary antibodies against brachyury (1:1,000; ab20680; Abcam, Cambridge, MA, USA) or β-actin (1:2,000; sc-130656; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. Membranes were washed with TBS and incubated with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (1:3,000; BA1011; Wuhan Boster Biological Technology, Ltd., Wuhan, China). Respective bands from these blots were observed using the BCIP/NBT Color Development Substrate (Promega Corporation). The experiments were repeated three times. The mean densities of the bands were analyzed using Quantity One 4.6.2 software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and normalized to that of β-actin.

Statistical analysis. Statistical analyses were performed using SPSS software, version 21.0 (IBM SPSS, Armonk, NY, USA) and GraphPad Prism, version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Differences in miR-219-5p expression were assessed using non-parametric tests (Mann-Whitney U test) due to the heterogeneity of variances of the residuals when applying analysis of variance. The relative luminescence ratios, ratio of cell proliferation and colony formation, and densities of bands were analyzed using Student's t-tests. Two-sided Fisher's exact test was used to identify differences between categorical variables. P<0.05 was considered statistically significant.

Results

miR-219-5p is downregulated in chordoma tissues and the U-CH2 cell line. Using miRanda and TargetScan, 16 miRNAs targeting brachyury mRNA were predicted, including miR-23a, miR-219-5p, miR-221, miR-222 and miR-448. Of these, RT-qPCR demonstrated that the expression of miR-219-5p was downregulated in chordoma tissue compared with normal skeletal muscle (Fig. 1A). Subsequently, the expression levels of miR-219-5p in U-CH2 cells were compared with CNE-2, Hep-2 and 293T cells (Fig. 1B). These results suggest that the expression of miR-219-5p is significantly decreased in chordoma tissues and the U-CH2 cell line.

miR-219-5p directly binds to the 3'-UTR of brachyury mRNA. The miR-219-5p seed sequence in the 3'-UTR of brachyury mRNA was predicted using miRanda (Fig. 2A). The wild-type and mutant T-3'UTR fragments, containing the miR-219-5p target site, were inserted into the reporter vectors and validated by DNA sequencing (Fig. 2B). Dual-luciferase reporter assays showed that, when miR-219-5p was overexpressed, the luciferase activity in the wild-type T-3'-UTR reporter vector was significantly reduced (Fig. 2C). However, luciferase activity in the reporter vector containing the mutant T-3'-UTR was not affected by miR-219-5p. These results suggest that miR-219-5p may negatively regulate brachyury expression by directly binding to the GACAAUC sequence in the 3'-UTR of T gene mRNA.
Effect of miR-219-5p overexpression on cell proliferation and colony formation in chordoma cells. To confirm the biological function of miR-219-5p in chordoma, CCK-8, EdU and clone formation assays of U-CH2 cells transfected with miR-219-5p mimics or NC were performed. As a comparison, the effect of miR-219-5p on proliferation and colony formation was also measured in 293T cells. Significantly reduced cell growth activity (P<0.01; Fig. 3A), colony formation (P<0.05;
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Fig. 3B and C) and proliferation (P<0.05; Fig. 3D and E) were observed in U-CH2 cells transfected with miRNA-219-5p mimics compared with NC. For 293T cells, there was no significant difference between the cells transfected with miR-219-5p mimics and NC in terms of colony formation (Fig. 3B and C) or cell proliferation (Fig. 3E). These results suggest that overexpression of miR-219-5p is able to suppress cell proliferation and colony formation in U-CH2 cells.

Effect of miR-219-5p knockdown on cell proliferation and colony formation in UCH-2 cells. The effect of inhibiting miR-219-5p on U-CH2 and 293T cells was examined. The results showed that downregulating the expression of miR-219-5p by transfection with miR-219-5p inhibitors significantly increased U-CH2 cell growth activity (P<0.05; Fig. 4A), colony formation (Fig. 4B and C), and proliferation (Fig. 4D and E), as compared with cells transfected with NC inhibitor. There was no significant difference in cell proliferation or colony formation between the 293T cells transfected with miRNA-219-5p inhibitor and NC inhibitor (Fig. 4). These results indicate that downregulation of miR-219-5p promotes U-CH2 cell proliferation and colony formation in vitro.

miR-219-5p regulates the expression of brachyury in U-CH2 cells. U-CH2 cells were transfected with miR-219-5p mimic, NC, NC inhibitor or miR-219-5p inhibitor, and the effects of miR-219-5p on the protein expression levels of brachyury were analyzed using western blotting (Fig. 5). The results demonstrated that the protein expression levels of brachyury were significantly reduced in miR-219-5p mimic-transfected U-CH2 cells compared with cells transfected with NC (P<0.01), but were increased in miR-219-5p inhibitor-transfected U-CH2 cells compared with cells transfected with NC inhibitor (P<0.05). These results suggest that miR-219-5p may regulate the protein expression of brachyury in U-CH2 cells.

miR-219-5p is correlated with tumor extent and recurrence. Among the 40 patients with skull base chordoma, 23 patients did not receive treatment prior to surgery. Five patients experienced tumor recurrence following the primary surgery and had to be operated on again. Twelve patients underwent the primary surgery at centers other than Xuanwu hospital. There was no significant relationship between the relative expression level of miR-219-5p and patient age (P=1.000) and gender (P=0.755; Table II). Compared with the initial tumors, the expression levels of miR-219-5p were significantly lower in recurrent tumors (P=0.022). The expression levels of miR-219-5p were downregulated in 12 of the 13 chordomas where the tumor had gone beyond the clivus. No statistically significant relationship was observed between surgical approaches and the miR-219-5p level (P=0.510).

Discussion

Owing to the intricate anatomic location, large tumor size and extensive bleeding during operation, complete surgical resection of chordoma is extremely difficult (2). A better understanding of the molecular mechanisms involved in
tumor progression may assist the development of novel therapeutic strategies for this malignant disease. Accumulating evidence indicates that miRNAs may have an important role in the progression of human chordoma (17-21). For example, reduced expression of miR-1 has been reported in chordoma, and miR-1 is suggested to suppress the growth of chordoma cells by inhibiting Met and histone deacetylase 4 (17). Further studies have shown that miR-1 inhibits chordoma cell migration and invasion and correlates with clinical prognosis (18,19). Another study confirmed that miR-608 and miR-34a were significantly downregulated in chordoma and may influence chordoma malignancy by targeting epidermal growth factor receptor (EGFR), B-cell lymphoma-xL and Met (20). Furthermore, some miRNAs are reported to impact the pathogenesis of chordoma by regulating the mitogen-activated protein kinase (MAPK) signaling pathway (21). In the present study, the relative expression level of miR-219-5p in 40 skull base chordoma specimens and different cell lines were evaluated. Our data suggested that miR-219-5p was downregulated in chordoma tissues and the U-CH2 cell line. In addition, the exogenous overexpression of miR-219-5p inhibited the proliferation and clonogenicity of U-CH2 cells by targeting brachyury, while suppression of miR-219-5p expression had the opposite effect.

Brachyury is a master regulator in chordoma that binds directly to ~100 targets and indirectly influences the expression of >60 other genes, including genes regulating the cell cycle, growth factors and cytokines, as well as extracellular matrix genes (22). Efforts have been made to clarify the mechanisms underlying the regulation of brachyury in chordoma. In the

![Figure 5. Western blot analysis of brachyury expression in U-CH2 cells following transfection with miR or miR inh. Brachyury expression was downregulated following transfection with miR (P<0.01) and upregulated after transfection with miR inh (P<0.05), as compared with NC and NC inh, respectively. miR, miR-219-5p mimics; miR inh, miR-219-5p inhibitor; NC inh, NC inhibitor.](image)

| Features               | miR-219-5p (n, %) |     |     |     | P-value |
|------------------------|-------------------|-----|-----|-----|---------|
|                        | Downregulation (<1) | Upregulation (>1) | Total (n) |
| Gender                 |                   |     |     |     |         |
| Male                   | 12 (57.1)         | 9 (42.9) | 21 |     |
| Female                 | 12 (63.2)         | 7 (36.8) | 19 |     |
| Age (years)            |                   |     |     |     |         |
| ≤40                    | 10 (58.8)         | 7 (41.2) | 17 |     |
| >40                    | 14 (60.9)         | 9 (39.1) | 23 |     |
| Tumor site             |                   |     |     |     |         |
| Clivus                 | 12 (44.4)         | 15 (55.6) | 27 |     |
| Beyond clivus          | 12 (92.3)         | 1 (7.7) | 13 |     |
| Initial/recurrent      |                   |     |     |     |         |
| Initial                | 10 (43.5)         | 13 (56.5) | 23 |     |
| Recurrent              | 14 (82.4)         | 3 (17.6) | 17 |     |
| Surgical approach      |                   |     |     |     |         |
| Endoscopic             | 18 (54.5)         | 15 (45.6) | 33 |     |
| Traditional            | 6 (85.7)          | 1 (14.3) | 7  |     |

Data are presented as n (%).
genera *Ascidians* and *Xenopus*, as well as zebrafish, brachyury has been shown to be regulated by fibroblastic growth factor receptors via the RAS/RAF/MAPK kinase/extracellular signal-regulated kinase-ETS2 signaling pathway, but genetic alterations of these genes have not been detected in human chordoma (23). The P63 gene can regulate brachyury expression in mouse embryonic fibroblasts and murine-derived osteosarcomas, but not in human chordomas (24). In the present study, the inhibitory effect of miR-219-5p on brachyury, predicted by miRanda and TargetScan, was validated by the dual-luciferase reporter assay. *In vitro* experiments showed that miR-219-5p regulated the protein expression of brachyury in chordoma cells, and affected cell proliferation and clonogenicity. The results of the present study suggest that the loss of regulation of miR-219-5p on brachyury may be an important mechanism in the initiation and progression of chordoma.

A review of the literature demonstrated that miR-219-5p expression is consistently dysregulated in various tumors (25-29). Downregulation of miR-219-5p has been reported in glioblastoma (25). The exogenous overexpression of miR-219-5p in glioma cell lines could inhibit cell proliferation, growth and migration by targeting EGFR and inhibiting the receptor tyrosine kinase pathway (26). In addition, low expression of miR-219-5p has been associated with higher clinical stages and recurrence rates of meningioma (27). The level of miR-219-5p was reduced in hepatocellular carcinoma. In a previous study, miR-219-5p inhibited cell proliferation *in vitro* and resulted in the arrest of the cell cycle at the GI/S transition by inhibiting the expression of glypican-3 (28). miR-219-5p was also downregulated in papillary thyroid carcinoma and had a critical role in cell growth by inhibiting estrogen receptor-α (29). These findings, together with the results of the present study, indicate that miR-219-5p may exert a tumor suppressor role in the carcinogenesis of various tumors.

It remains controversial as to whether brachyury is a prognostic indicator for chordoma. Kitamura *et al.* (30) reported that the expression of brachyury was an independent prognostic indicator in skull base chordoma, and was associated with a poor prognosis. Zhang *et al.* (31) analyzed a chordoma tissue microarray and determined that brachyury was not a prognostic indicator for chordoma, although the specimens were mainly derived from mobile spine or sacrum chordoma. Expression of miR-219-5p has been correlated with the recurrence rate of meningioma (27) and overall survival time of hepatocellular carcinoma patients (28). The present study demonstrated that miR-219-5p expression was associated with tumor extent and recurrence, although no definitive conclusion could be drawn owing to the small sample size of the present study. As a regulator of brachyury, the prognostic value of miR-219-5p in skull base chordoma requires further investigation.

In summary, the present study identified that miR-219-5p was downregulated in chordoma tissues and the U-CH2 cell line, and was associated with extensive tumor and recurrence. Furthermore, miR-219-5p exhibited the ability to inhibit cell proliferation and clonogenicity of chordoma by targeting brachyury. These findings suggested that miR-219-5p may act as a tumor suppressor in human chordoma, and it may be a potential target for therapeutic intervention in chordoma.

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