**MicroRNA-362 Inhibits Cell Proliferation and Invasion by Directly Targeting SIX1 in Colorectal Cancer**

Jin’e Wan¹, Jian Yang², Cuixia Qiao², Xiaomei Sun³, Aiting Di³, Lize Zhang³, Dandan Wang³, and Gang Zhao³

¹Departments of Hyperbaric Oxygen and ³Anorectal Surgery, The Affiliated Hospital of Qingdao University, Qingdao; ²Department of Oncology, Zouping Centre Hospital, Binzhou, China.

**Purpose:** Colorectal cancer (CRC) is the third most common cancer in China and poses high morbidity and mortality. In recent years, increasing evidence has indicated that microRNAs played important functions in the occurrence and development of tumors. The purpose of this study was to identify the biological mechanisms of miR-362 in CRC.

**Materials and Methods:** Quantitative real-time PCR was carried out to assess the expression of miR-362 and SIX1. The Kaplan-Meier method was employed to evaluate the 5-year overall survival of CRC patients. The proliferative and invasive abilities of CRC cells were assessed by MTT and transwell assays.

**Results:** miR-362 was significantly decreased in CRC tissues and cell lines, compared to the normal tissues and normal cells. A significant connection was confirmed between the overall survival of 53 CRC patients and low expression of miR-362. Downregulation of miR-362 inhibited the proliferation and invasion through binding to the 3'-UTR of SIX1 mRNA in CRC. Additionally, we discovered that SIX1 was a direct target gene of miR-362 and that the expression of miR-362 had a negative connection with SIX1 expression in CRC. SIX1 could reverse partial functions in the proliferation and invasion in CRC cells.

**Conclusion:** miR-362 may be a prognostic marker in CRC and suppress CRC cell proliferation and invasion in part through targeting the 3'-UTR of SIX1 mRNA. The newly identified miR-362/SIX1 axis provides insight into the progression of CRC.

**Key Words:** miR-362, SIX, proliferation, invasion, colorectal cancer

---

**INTRODUCTION**

With approximately 600000 deaths every year, colorectal cancer (CRC) accounts for 10% of all cancer cases and mortalities, making CRC the third leading cause of cancer deaths.¹,² The clinic outcomes of CRC patients are poor because of high rates of local recurrence and distant metastasis.³,⁴ Therefore, identifying tumor molecular markers for metastasis and recurrence in patients with CRC is critical.
MATERIALS AND METHODS

Tissue samples
Cancer and matched paracancerous tissues were obtained from CRC patients who were diagnosed in the Department of Anorectal Surgery in the Affiliated Hospital of Qingdao University from January 2016 to June 2018. The clinical information of the patients and the associations between the expression of miR-362 and the clinicopathological features are shown in Table 1. The fresh tissues were instantly frozen in liquid nitrogen and stored at -80°C. The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University, and all the patients provided informed consent before specimen collection.

Table 1. miR-362 Expression and Clinicopathological Features in 53 Colorectal Cancer Patients

| Clinicopathological features | Cases (n=53) | miR-362 expression | p value |
|-----------------------------|-------------|--------------------|---------|
| Age (yr)                    |             |                    |         |
| >50                         | 24          | 13 (54.2)          | 11 (45.8) | 0.353 |
| ≤50                         | 29          | 12 (41.4)          | 17 (56.8) |
| Sex                         |             |                    |         |
| Male                        | 30          | 17 (56.7)          | 13 (43.3) | 0.114 |
| Female                      | 23          | 8 (34.8)           | 15 (65.2) |
| Tumor size (mm)             |             |                    |         |
| >5.0                        | 27          | 16 (59.3)          | 11 (40.7) | 0.072 |
| ≤5.0                        | 26          | 9 (36.4)           | 17 (65.4) |
| TNM stage                   |             |                    |         |
| III–IV                      | 26          | 16 (61.5)          | 10 (38.5) | 0.040* |
| I–II                        | 27          | 9 (33.3)           | 18 (66.7) |
| Lymph-node metastasis       |             |                    |         |
| >2                          | 30          | 18 (60.0)          | 12 (40.0) | 0.033* |
| 0–2                         | 23          | 7 (30.4)           | 16 (69.6) |
| Tumor location              |             |                    |         |
| Left-side                   | 28          | 17 (60.7)          | 11 (39.3) | 0.037 |
| Right-side                  | 25          | 8 (32.0)           | 17 (68.0) |

Variables are expressed as a number (percentage). *p<0.05 (p values are calculated with chi-square test).
was added to each well and incubated for 4 h at 37°C. Next, the medium was removed, and 150 μL of dimethyl sulfoxide (DMSO; Sigma-Aldrich) were added to dissolve the formazan. Absorbance was measured at 490 nm using an automatic multi-well spectrophotometer (Bio-Rad, Richmond, CA, USA). All experiments were performed in triplicate.

**Transwell assays**

Transwell chambers (8-μm; Millipore, Billerica, MA, USA) covered with Matrigel (BD Biosciences, San Jose, CA, USA) were utilized to carry out the invasion assay. SW480 cells resuspended in serum-free medium were placed into the upper chamber transwell insert. Meanwhile, the lower chamber was filled with normal medium containing 20% FBS as the chemoattractant. Subsequently, the non-invaded cells were wiped off by a cotton swab, while the invasive cells were fixed and stained with 100% methanol and 0.5% crystal violet solution, in that order. Cells were then counted using a microscope (CX31; Olympus, Tokyo, Japan). Each experiment was repeated at least three times.

**Dual-luciferase reporter assay**

The wild type 3’-UTR fragment of the SIX1 mRNA that contained the complementary sequences of miR-362 or the mutant sequences were inserted into pmirGlo vector (Promega, Madison, WI, USA), which were confirmed by sequencing. The miR-362 mimic or negative control and the pmirGlo constructs were co-transfected into SW480 cells using the Lipofectamine 2000 transfection reagent (Invitrogen), according to the manufacturer’s instructions. The cells were lysed after calculated at 48 h, and the Dual-Luciferase Reporter Assay kit (Promega) was used to calculate the luciferase activity, according to the manufacturer’s instructions. The firefly luciferase activity was normalized to Renilla luciferase activity.
Statistical analysis
All data were analyzed by SPSS 19.0 software (IBM Corp., Armonk, NY, USA) and expressed as the mean±standard error. Two-sided Student’s t-tests or one-way analysis of variance was employed to compare with differences between two groups or more than two groups. p values <0.05 were considered to indicate a statistically significant difference.

RESULTS
miR-362 expression reduced in CRC and downregulation of miR-362 predicts poor prognosis
The miR-362 mRNA levels in 53 pairs of CRC and adjacent normal tissues were assessed by RT-qPCR in an attempt to determine the importance of miR-362. As expected, we discovered that the expression of miR-362 was higher in CRC than in adjacent normal tissues (p<0.001) (Fig. 1A). Furthermore, the overall survival of all 53 patients was assessed, and Kaplan-Meier analysis indicated that miR-362 downregulation elicited shorter overall survival than miR-362 upregulation in CRC patients (p=0.048) (Fig. 1B).

The expressions of miR-362 in two human CRC cell lines (LOVO and SW480) and normal colon cells (CCD-18Co) were also evaluated using RT-qPCR. In comparison with the normal CCD-18Co colon cells, the expression of miR-362 was lower in LOVO (p=0.002) and SW480 CRC cells (p<0.001) (Fig. 1C). To investigate the biological mechanisms of miR-362 in CRC, miR-362 mimic or miR-362 inhibitor was injected into SW480 cells, and the transfection efficiency was evaluated by qRT-PCR. As shown in Fig. 1D, the mRNA levels of miR-362 increased in miR-362 mimic-transfected cells (p<0.001) and decreased in miR-362 inhibitor-transfected SW480 cells (p=0.006).

We also assessed associations between miR-362 expression and clinicopathological features in CRC. As shown in Table 1, the expression of miR-362 held significant correlations with TNM stage (p=0.040), lymph node metastasis (p=0.033), and
tumor location \((p=0.037)\). Meanwhile, miR-362 tended to exhibit a negative correlation with tumor size \((p=0.072)\). However, there were no significant correlations between the expression of miR-362 and age \((p=0.353)\) or sex \((p=0.114)\) (Table 1).

miR-362 suppresses cell proliferation and invasion
To elucidate the roles of miR-362 in CRC, MTT and transwell assays were performed to evaluate proliferative and invasive abilities. MTT data indicated that cell viability decreased \((p=0.038, p=0.007, \text{and } p=0.002 \text{ of 48 h, 72 h, and 96 h})\) in miR-362 mimic-transfected SW480 cells, while the opposite was observed in miR-590 inhibitor-transfected cells \((p=0.027, p=0.003, \text{and } p<0.001 \text{ of 48 h, 72 h, and 96 h})\) (Fig. 2A). Similar with the MTT results, transwell assay revealed that miR-362 mimic reduced \((p<0.001)\) cell invasion, while miR-362 inhibitor promoted \((p<0.001)\) invasive ability in SW480 cells (Fig. 2B).

SIX1 upregulated in CRC and overexpression of SIX1 predicts poor prognosis
The expression of SIX1 was calculated by RT-qPCR in CRC tissues and cell lines. As expected, the expression of SIX1 showed significant increases in CRC tissues, compared to the paracancerous tissues \((p<0.001)\) (Fig. 3A). Therefore, the associations between the expression of miR-362 and SIX1 were assessed, and we discovered that miR-362 had a negative relationship with the expression of SIX1 in CRC tissues \((r=-0.525, p<0.001)\) (Fig. 3B). Moreover, Kaplan-Meier analysis revealed that upregulation of SIX1 predicts poor 5-year overall survival in CRC patients \((p=0.021)\) (Fig. 3C). In addition, the expression of SIX1 in CRC cells LOVO and SW480 and normal colon cell CCD-18Co was evaluated by RT-qPCR. We discovered that the expression of SIX1 was higher in LOVO and SW480 cells than in CCD-18Co cells \((p=0.004 \text{ and } p<0.001)\) (Fig. 3D).

**Fig. 3.** SIX1 was upregulated in CRC, and overexpression of SIX1 predicted poor prognosis. (A) The expression of SIX1 reflected significant increases in CRC tissues, compared to paracancerous tissues (PT). (B) miR-362 exhibited negative correlations with the expression of SIX1 in CRC tissues. (C) Upregulation of SIX1 predicted poor 5-year overall survival in CRC patients. (D) The expression of SIX1 was higher in LOVO and SW480 cells, compared with CCD-18Co cells. **\(p<0.01\), ***\(p<0.001\). CRC, colorectal cancer.
SIX1 is a target of miR-362 and expression of SIX1 is mediated by miR-362
TargetScan was employed to predict the potential target genes of miR-362, and SIX1 was discovered to be a direct target of miR-362, showing putative binding sequences. To investigate whether miR-362 directly binds to the 3’-UTR of SIX1 mRNA, the putative binding sequences on SIX1 mRNA were mutated, as shown in Fig. 4A. Luciferase reporter assay was applied to identify the prediction, and the luciferase ability of the wild type of SIX1 3’-UTR was notably decreased (p=0.002) by miR-362 mimic, but had no effect (p=0.800) on the mutant type of SIX1 3’-UTR (Fig. 4B). Moreover, after transfecting SW480 cells with miR-362 mimic or miR-362 inhibitor, we evaluated the expression of SIX1, the results of which indicated that the mRNA levels of SIX1 were reduced in miR-362 mimic-transfected cells (p=0.005). On the contrary, SIX1 mRNA levels were increased when miR-362 was knocked down in SW480 cells (p=0.002) (Fig. 4C).

Knockdown of SIX1 inhibits the proliferation and invasion of SW480 cells
To explore the functions of SIX1 in CRC, the siRNA-SIX1 was utilized to knockdown SIX1 in SW480 cells detected by RT-qPCR (p<0.01) (Fig. 5A). MTT and transwell assays were performed to calculate the proliferative and invasive abilities after knockdown of SIX1. In comparison with siRNA-NC, MTT data indicated that cell viability was decreased by transfecting siRNA-SIX1 in SW480 cells (p<0.01) (Fig. 5B). Similarly, transwell assay revealed that siRNA-SIX1 reduces (p<0.01) the invasive ability of SW480 cells (Fig. 5C).

SIX1 could reverse the effects of miR-362 on cell proliferation and invasion
In an attempt to further explore the biological mechanisms of miR-362 in cell proliferation and invasion, rescue experiment was performed to verify the results above. pcDNA3.1-SIX1 was utilized to re-express SIX1 in miR-362 mimic-transfected SW480 cells, and the transfection efficiency was detected by RT-qPCR, as shown in Fig. 6A (p=0.011). The proliferative and invasive capacities were assessed by MTT or transwell assays. As expected, the proliferative ability was increased by re-expressing SIX1 in miR-362 mimic transfected SW480 cells (p=0.045) (Fig. 6B). Similar with the results of MTT, the transwell assay results demonstrated that the invasive ability was enhanced by transfection of pcDNA3.1-SIX1 in SW480 cells in which miR-362 was overexpressed (p=0.030) (Fig. 6C).

DISCUSSION
CRC is a frequent malignant tumor with high metastasis and recurrence, and rates thereof are increasing every year. Therefore, identifying tumor molecular markers for metastasis and recurrence is essential in the diagnosis of CRC patients.
miRNAs have been reported to act as a tumor promoter or suppressor in various cancers. Zou, et al. revealed that miR-362 functions as a tumor suppressor by reducing cell proliferation, migration, invasion, and cell cycle and by enhancing cell apoptosis in renal cancer. Similarly, Wu, et al. indicated that miR-362 suppresses the proliferation and migration of neuroblastoma cells. Consistent with these findings, we discovered that miR-362 was downregulated in CRC tissues and cell lines. Moreover, the CRC patients in the low miR-362 group showed poorer overall survival than patients in the high miR-362 group, consistent with the findings of Kheirollahi, et al. in glioma. Ni, et al. revealed that miR-362 repressed proliferation and metastasis in breast cancer. Consistent with the findings mentioned above, we deemed that overexpression of miR-362 suppressed proliferation and invasion in the CRC cell line SW480. In reverse, the cell proliferative and invasive capacities were enhanced by miR-362 inhibitor.

SIX1 was reported to enhance the proliferation and migration of osteosarcoma cells. Similarly, SIX1 was found to enhance cell proliferation and tumorigenesis through PI3K/AKT pathway in osteosarcoma. Moreover, Sun, et al. indicated that SIX1 regulated cell motility, tumor metastasis, and EMT in cervical cancer. Consistent with the findings above, we revealed that SIX1 was upregulated in CRC tissues and cell lines. Also, upregulation of SIX1 predicted shorter overall survival than SIX1 downregulation, which was consistent with previously reported findings in pancreatic ductal adenocarcinoma, gastric adenocarcinoma, hepatocellular carcinoma, and esophageal squamous cell carcinoma. In addition, SIX1 was a target gene of several miRNAs, including miR-204, miR-30a, miR-188 and miR-27a. We proposed that miR-362 targets and regulates the expression of SIX1. Moreover, knockdown of SIX1 had the same effect on the proliferation and migration as transfection of miR-362 mimic. SIX1 reversed the functions of miR-362 on cell proliferation and invasion in CRC.

**Fig. 5.** Knockdown of SIX1 inhibited the proliferation and invasion of SW480 cells. (A) The siRNA-SIX1 was conducted to knockdown SIX1 in SW480 cells. (B) In comparison with siRNA-NC, the cell viability was decreased by transfecting siRNA-SIX1 in SW480 cells. (C) Transwell assay revealed that siRNA-SIX1 reduces the invasive ability in SW480 cells (0.5% crystal violet, ×200). **p<0.01.
In conclusion, miR-362 was significantly decreased in CRC tissues and cell lines, compared to normal tissues and cells. We confirmed a significant association between the overall survival of 53 CRC patients and low expression of miR-362. Downregulation of miR-362 inhibited cell proliferation and invasion through SIX1 in CRC. Additionally, we also discovered that SIX1 was a direct target gene of miR-362 and that the expression of miR-362 had negative correlations with SIX1 in CRC. SIX1 could partially reverse the functions of miR-362 on proliferation and invasion in CRC cells.

AUTHOR CONTRIBUTIONS

Conceptualization: Gang Zhao. Data curation: Jin’e Wan and Jian Yang. Formal analysis: Jian Yang and Cui Xia Qiao. Funding acquisition: Gang Zhao. Investigation: Xiaomei Sun. Methodology: Jin’e Wan and Aiting Di. Project administration: Gang Zhao. Resources: Gang Zhao. Software: Lize Zhang. Supervision: Cui Xia Qiao and Dandan Wang. Validation: Jian Yang. Visualization: Gang Zhao. Writing—original draft: Jin’e Wan. Writing—review & editing: Gang Zhao and Cui Xia Qiao.

ORCID iDs

Jin’e Wan https://orcid.org/0000-0002-5196-2344
Jian Yang https://orcid.org/0000-0001-5772-151X
Cui Xia Qiao https://orcid.org/0000-0002-7406-6542
Xiaomei Sun https://orcid.org/0000-0002-8590-7466
Aiting Di https://orcid.org/0000-0002-2462-6927
Lize Zhang https://orcid.org/0000-0002-8406-8656
Dandan Wang https://orcid.org/0000-0002-3628-9569
Gang Zhao https://orcid.org/0000-0002-2315-2045

REFERENCES

1. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet 2014;383:1490-502.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
3. Shimada H, Tanaka K, Endou I, Ichikawa Y. Treatment for colorec-
miR-362 Inhibited Colorectal Cancer Progress

https://doi.org/10.3349/ymj.2019.60.5.414

1. Talimental liver metastases: a review. Langenbecks Arch Surg 2009;394: 973-83.
2. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
3. Lee YS, Dutta A. MicroRNAs in cancer. Annu Rev Pathol 2009;4: 199-227.
4. Ju J. Implications of miRNAs in colorectal cancer chemoresistance. Int Drug Discov 2011;2011:2063.
5. Ke SB, Qiu H, Chen JM, Shi W, Chen YS. MicroRNA-202-3p functions as a tumor suppressor in colorectal carcinoma by directly targeting SMARCC1. Gene 2018;676:329-35.
6. Chen E, Li Q, Wang H, Zhang P, Zhao X, Yang E, et al. MiR-32 promotes tumorigenesis of colorectal cancer by targeting BMP5. Biomed Pharmacother 2018;106:1046-51.
7. Wang D, Wang H, Li Y, Li Q. MiR-362-3p functions as a tumor suppressor through targeting MCM5 in cervical adenocarcinoma. Biosci Rep 2018;38:BSR20180668.
8. Yang P, Ni F, Deng BQ, Qiang G, Zhao H, Yang MZ, et al. MiR-362-5p promotes the malignancy of chronic myelocytic leukemia via down-regulation of GADD45a. Mol Cancer 2015;14:190.
9. Xia JT, Chen LZ, Jian WH, Wang KB, Yang YZ, He WL, et al. MicroRNA-362 induces cell proliferation and apoptosis resistance in gastric cancer by activation of NF-kB signaling. J Transl Med 2014;12:33.
10. Wang N, Feng Y, Xu J, Zou J, Chen M, He Y, et al. miR-362-3p regulates cell proliferation, migration and invasion of trophoblastic cells under hypoxia through targeting Pax3. Biomed Pharmacother 2018;99:462-8.
11. Christensen LL, Tobiasen H, Holm A, Schecketer T, Ostenfeld MS, Thorsen K, et al. MRNA-362-3p induces cell cycle arrest through targeting of E2F1, USF2 and PTPN1 and is associated with recurrence of colorectal cancer. Int J Cancer 2013;133:67-78.
12. Christensen KL, Patrick AN, McCoy EL, Ford HL. The six family of homeobox genes in development and cancer. Adv Cancer Res 2008;101:93-126.
13. Kumar JP. The sine oculis homeobox (SIX) family of transcription factors as regulators of development and disease. Cell Mol Life Sci 2009;66:565-83.
14. Wu W, Ren Z, Liu H, Wang L, Huang R, Chen J, et al. Core promoter analysis of porcine SIX1 gene and its regulation of the core promoter activity by CpG methylation. Gene 2013;529:238-44.
15. Micalezzi DS, Wang CA, Farabaugh SM, Schiemann WP, Ford HL. Homeoprotein SIX1 increases TGF-beta type I receptor and core promoter activity by CpG methylation. Gene 2013;529:238-44.
16. Xue X, Li Y, Yang X. SIX1 is overexpressed in endometrial carcinoma and promotes the malignant behavior of cancer cells through ERK and AKT signaling. Oncol Lett 2016;12:3435-40.
17. Lerbs T, Bish T, Scholte S, Pecqueux M, Kristiansen G, Schneider M, et al. Inhibition of SIX1 affects tumour invasion and the expression of cancer stem cell markers in pancreatic cancer. BMC Cancer 2017;17:249.
18. Zhang X, Xu R. Six1 expression is associated with a poor prognosis in patients with glioma. Oncol Lett 2017;13:1293-8.
19. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
20. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China. 2015. CA Cancer J Clin 2016;66:115-32.
21. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-8.
22. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257-61.
23. Zou X, Zhong J, Li J, Su Z, Chen Y, Deng W, et al. miR-362-3p targets nemo-like kinase and functions as a tumor suppressor in renal cancer cells. Mol Med Rep 2016;13:994-1002.
24. Wu K, Yang L, Chen J, Zhao H, Wang J, Xu S, et al. MiR-362-5p inhibits proliferation and migration of neuroblastoma cells by targeting phosphatidylinositol-3-kinase-C2z. FEBS Lett 2015;589:1911-9.
25. Kheirrollahi M, Moodi M, Ashouri S, Nikpour P, Kazemi M. Evaluation of miR-362 expression in astrocytoma of human brain tumors. Adv Biomed Res 2017;6:128.
26. Ni F, Gu Z, Guo Q, Hu Z, Wang X, Chen D, et al. Downregulation of miR-362-5p inhibits proliferation, migration and invasion of human breast cancer MCF7 cells. Oncol Lett 2016;11:1155-60.
27. Hua L, Fan L, Aichun W, Yongqin Z, Qingqing C, Xiaojian W. Inhibition of Six1 promotes apoptosis, suppresses proliferation, and migration of osteosarcoma cells. Tumour Biol 2014;35:1925-31.
28. Yu C, Zhang B, Li YL, Yu XR. SIX1 reduces the expression of PTEN via activating PI3K/AKT signal to promote cell proliferation and tumorigenesis in osteosarcoma. Biomed Pharmacother 2018;105:10-7.
29. Sun SH, Liu D, Deng YT, Zhang XX, Wan DY, Xi BX, et al. SIX1 coordinates with TGFβ signals to induce epithelial-mesenchymal transition in cervical cancer. Oncol Lett 2016;12:1271-8.
30. Jin A, Xu Y, Liu S, Jin T, Li Z, Jin H, et al. Sineoculis homeobox homolog 1 protein overexpression as an independent biomarker for pancreatic ductal adenocarcinoma. Exp Mol Pathol 2014;96:54-60.
31. Lv H, Cai A, Sun F, Zhang Y, Li Y, Li L, et al. Sineoculis homeobox homolog 1 protein as an independent biomarker for gastric adenocarcinoma. Exp Mol Pathol 2014;97:74-80.
32. Kong J, Zhou X, Liu S, Jin T, Piao Y, Liu C, et al. Overexpression of sineoculis homeobox homolog 1 predicts poor prognosis of hepatocellular carcinoma. Int J Clin Exp Pathol 2014;7:3018-27.
33. He Z, Li G, Tang L, Li Y. SIX1 overexpression predicts poor prognosis and induces radiosensitivity through AKT signaling in esophageal squamous cell carcinoma. Onco Targets Ther 2017;10:1071-9.
34. Xia Y, Zhu Y, Ma T, Pan C, Wang J, He Z, et al. miR-204 functions as a tumor suppressor by regulating SIX1 in NSCLC. FEBS Lett 2014;583:3703-12.
35. O’Brien JH, Hernandez-Lagunas L, Artinger KB, Ford HL. MicroRNA-30a regulates zebrafish myogenesis through targeting the transcription factor Six1. J Cell Sci 2014;127(Pt 10):2291-301.
36. Wang L, Liu H. microRNA-188 is downregulated in oral squamous cell carcinoma and inhibits proliferation and invasion by targeting SIX1. Tumour Biol 2016;37:1105-13.
37. Towers CG, Guarnieri AL, Micalezzi DS, Harrell JC, Gillen AE, Kim J, et al. The Six1 oncoprotein downregulates p53 via concomitant regulation of RPL26 and microRNA-27a-3p. Nat Commun 2015;6:10077.