miR-877 inhibits the proliferation, migration, and invasion of osteosarcoma cells by targeting gamma-glutamylcyclotransferase

Chenguang Jia *, Jianguo Gao *, Lianbo Wang, Zhuo Li, Zhaoliang Dong, Liming Yao and Xiaowei Yao

Department of Orthopedics, the Chest Hospital of Hebei Province, Shijiazhuang 050041, China

Abstract. Gamma-glutamylcyclotransferase (GGCT) can promote the progression of osteosarcoma (OS). MicroRNAs also play significant roles in regulating the progression of OS. This study was designed to investigate whether miR-877 exerts its function in OS by targeting GGCT. The proliferation of OS cells (Saos-2 and U2OS) was detected by MTT and colony formation assays. The migration and invasion of OS cells were detected by transwell assays. The expressions of miRNAs and GGCT were detected by quantitative real-time PCR and Western blot. The luciferase reporter assay was performed to assess whether miR-877 could target GGCT. miR-877 was down-regulated both in OS tissues and OS cell lines (Saos-2 and U2OS). The overexpression of miR-877 inhibited the proliferation, migration, and invasion of OS cell lines, while the knockdown of miR-877 could negate effects. The expression of GGCT was increased in Saos-2 and U2OS cells. miR-877 could target GGCT, and the mRNA level of GGCT in Saos-2 and U2OS cells was decreased by the overexpression of miR-877. miR-877 overexpression inhibited the migration and invasion and suppressed the proliferation of Saos-2 and U2OS cells, and the overexpression of GGCT reversed this effects. The knockdown of miR-877 promoted the migration and invasion and facilitated the proliferation of Saos-2 and U2OS cells, and the silence of GGCT abolished this effects. Our findings suggested that miR-877 could inhibit the proliferation, migration, and invasion of OS cells by targeting GGCT.

Key words: miR-877, Osteosarcoma, Gamma-glutamylcyclotransferase, Invasion, Migration

OSTEOSARCOMA (OS), the most common primary malignant tumor of bone, mainly occurred in adolescents and young adults [1]. Although its incidence is very low, OS has a high mortality rate due to its high rate of metastasis [2]. At present, although the survival time of OS patients has increased with the improvement of the treatment strategies such as systemic chemotherapy and surgical excision, the 5-year survival rate is still not ideal because of the pathogenesis of OS is still not fully elucidated [3-5]. Thus, an in-depth study of molecular mechanisms that participated in OS may provide novel therapeutic targets and strategies for OS.

Gamma-glutamylcyclotransferase (GGCT), also known as C7orf24 and CRF21, is one of the main enzymes of glutathione metabolism, catalyzing the conversion of γ-Glu-AA to pyroglutamate [6, 7]. GGCT is widely expressed in bacteria, plants, nematodes, and other species, and is highly conserved in species [8]. It has been reported that GGCT is up-regulated in several types of cancers, and the depletion of GGCT can exert anti-cancer function in these cancers, including prostate cancer, esophageal squamous carcinoma, breast cancer, gastric cancer, and ovarian cancer [9-13]. Also, GGCT plays a crucial role in the OS. Uejima et al. pointed out that GGCT is up-regulated in OS cell lines and primary tumor samples, which could be considered as a biomarker of OS. Their study also showed that the silence of GGCT with siRNA suppresses the migration, invasion and proliferation of OS cells [14]. Nevertheless, the upstream regulatory mechanism of GGCT in OS remains unknown.

MicroRNAs (miRNAs), 18–25 nt in length, are classes of endogenous non-coding small RNAs [15]. It has been well established that miRNAs can bind to the 3'-untranslated region (3'-UTR) of target mRNAs. The expressions of miRNAs can be inhibited by contributing to mRNA degradation or blocked mRNA translation [15-17]. It has been proven that many miRNAs are aberrantly expressed in OS, which are closely related with the development and progression of OS, such as miR-140-5p, miR-708, miR-1248 and miR-1236-3p.
According to the data mining analyses of the microarray datasets of OS from NCBI GEO public databases (GSE28425 and GSE28425), we found that miR-877 is one of the differentially expressed miRNAs in OS. Based on the bioinformatics analysis by the ENCORI online software, there was a predicted binding site of miR-877 on GGCT 3'-UTR. Therefore, we speculated that miR-877 plays its key function in OS by targeting GGCT.

In this study, we aimed to investigate whether miR-877 exerts its function in OS by targeting GGCT. As expected, our results suggested that miR-877 was down-regulated in OS and then inhibited the proliferation, migration, and invasion of OS cells by targeting GGCT.

Materials and Methods

Clinical samples
OS tissues and the adjacent bone tissues were both collected from patients with OS who underwent tumor resection in our center. Patients in this study have signed informed consent.

Cell culture
Normal human osteoblasts (hFOB) and OS cell lines (Saos-2 and U2OS) were both obtained from ATCC (USA), which were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco, USA) medium containing 10% fetal bovine serum (FBS, Gibco, USA), 100 mg/mL streptomycin and 100 U/mL penicillin at 37°C and 5% CO₂.

Cell transfection
The overexpression vectors (miR-877 mimic and pcGGCT) and silence vectors (miR-877 inhibitor and sh-GGCT) were synthesized by GenePharma (China). After Saos-2 and U2OS cells were seeded in the 24-well plates and incubated for 24 h, they were transfected with vectors using Lipofectamine 2000 (Invitrogen, USA).

MTT assay
Saos-2 and U2OS cells were seeded in the 96-well plate (5 × 10³ cells/well), 50 μL MTT reagent was added into each well and then incubated for 3 h. After cells were centrifuged at 1,000 × g at 4°C for 5 min, the supernates were removed. Later, 150 μL MTT solvent was added into each well. To assess cell proliferation, the absorbance at 590 nm was detected by a microplate reader (Thermo Labsystems, USA) after incubated for 15 min.

Cell migration assay
The migration of Saos-2 and U2OS cells were assessed by transwell assay. In brief, the upper transwell chambers was added in 300 μL serum-free DMEM medium, and 500 μL DMEM medium with FBS was added into the lower transwell chambers. Then 2 × 10⁴ cells were seeded into the upper transwell chambers and incubated for 48 h. After transwell chambers were taken out, in the lower transwell chambers were added into 5% paraformaldehyde to fix the cells for 20 min and then with added into 0.1% crystal violet to stain the cells for 20 min. Then cells were observed by using a microscope (Leica, Japan).

Cell invasion assay
Transwell assay was performed to assess the invasion of Saos-2 and U2OS cells. Firstly, the upper transwell chambers were pre-coated with 70 μL Matrigel (3.9 μg/μL). Secondly, serum-free DMEM medium (300 μL) was added into the upper transwell chambers and the FBS-contained DMEM medium (500 μL) was added into the lower transwell chambers. Thirdly, 2 × 10⁴ cells were seeded into the upper transwell chambers and incubated for 48 h. Next, transwell chambers were taken out and added into 5% paraformaldehyde to fix the cells for 20 min and then added into 0.1% crystal violet to stain the cells for another 20 min. Finally, cells were observed by using a microscope (Leica, Japan).

Colony formation assay
Saos-2 and U2OS cells were suspended in the DMEM medium supplemented with FBS. Then cells were seeded into culture dishes at 37°C and 5% CO₂ for 2 weeks. After the supernates were removed, cells were washed with PBS, fixed with 4% paraformaldehyde and stained with crystal violet, which observed under a microscope (Leica, Japan).

Luciferase reporter assay
The sequences of GGCT 3'-UTR wild type (WT) and GGCT 3'-UTR mutated (Mut) were cloned into plasmid vector expressing luciferase. Each recombinant vector was co-transfected into HEK-293T cells with miR-877 mimic or NC. Then the luciferase activity was detected.

Real-time quantitative PCR
The total RNA was isolated from clinical samples and OS cells by using Trizol Reagent (Invitrogen). The quality and concentration of RNA were evaluated by a spectrophotometer. cDNA was synthesized by 500 ng RNA of each sample using Hi-Fi cDNA Synthesis Kit (Abcam). According to the manufacturer’s instruction of SYBR Green PCR Master Mix Kit (Applied Biosystem, USA), the real-time quantitative PCR (qRT-PCR) was performed. The relative expressions of genes were calculated using 2⁻ΔΔCT method. U6 was used as an internal
reference of miRNAs and GAPDH was utilized as an internal reference of GGCT.

**Western blot**
The OS cells were lysed by RIPA lysis supplemented with proteinase inhibitor to extract protein. The concentration of protein samples was detected using BCA Protein Quantification (Abcam), and 30 μg protein of each sample was used to perform SDS-PAGE. After that, the protein was transferred onto the polyvinylidene fluoride (PVDF) membranes, which were incubated with the primary antibodies: anti-GGCT (Abcam, 1:1,000) and anti-GAPDH (Abcam, 1:2,000) at 4°C overnight. Then they were incubated with the secondary antibody at room temperature for 1.5 h. The protein bands were visualized using BeyoECL Plus Kit (Beyotime, China).

**Statistical analysis**
Data analysis was performed using GraphPad Prism 7.0 software. All data were expressed as mean ± standard deviation (SD). The difference between groups was compared using Student’s t-test or one-way analysis of variance (ANOVA) where applicable.

**Results**
miR-877 downregulated in OS and inhibited the viability of OS cells
We used the microarray datasets of OS from NCBI GEO public databases (GSE28425 and GSE28425) and conducted a data mining analysis to select the differentially expressed miRNAs ($p < 0.05$, logFC < -0.4) in OS. The Venn diagrams of the two integrated analyses showed that 31 miRNAs were significantly and consistently differentially expressed in OS (Fig. 1A). We then selected 4 miRNAs whose roles in OS have not been reported of these 31 miRNAs, and the expressions of these 4 miRNAs in OS were verified by qRT-PCR.

**Fig. 1** The expression of miR-877 in OS and the effect of miR-877 on the proliferation of OS cells. (A) Venn diagram showed the numbers of differentially expressed miRNAs in OS that were overlapped in GSE28425 and GSE69470 databases. (B) The expressions of miRNAs in OS tissues and cell lines (U2OS and Saos-2) were detected by qRT-PCR. (C–H) U2OS and Saos-2 cells were transfected with miR-877 mimic or miR-877 inhibitor. (C and F) The expression of miR-877 was detected by qRT-PCR. (D–E and G–H) The proliferation of U2OS and Saos-2 cells were detected by MTT assay. NC, negative control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. 
Among these 4 miRNAs, the expression of miR-877 was changed in Saos-2 and U2OS cells to the largest extent (Fig. 1B). Meanwhile, the expression of miR-877 was significantly decreased in clinical OS tissues compared with the adjacent tissues. Moreover, we overexpressed or silenced miR-877 in Saos-2 and U2OS cells (Fig. 1C and F) to detect the viability of cells. As shown in Fig. 1D and E, the overexpression of miR-877 decreased the viability of Saos-2 and U2OS cells. On the contrary, the knockdown of miR-877 increased the viability of Saos-2 and U2OS cells (Fig. 1G and H). In addition, we found that the intervention of miR-633 and miR-574-5p had no effect on the MTT results of OS cell lines (Fig. S1). Thus, we explored the key role of miR-877 in OS.

**miR-877 suppressed the migration, invasion and proliferation of OS cells**

Next, we assessed the influence of miR-877 overexpression and silence on the migration, invasion and proliferation of OS cells. Results from the colony formation assay revealed that miR-877 overexpression reduced the colony numbers of Saos-2 and U2OS cells, while its knockdown increased the colony numbers of Saos-2 and U2OS cells (Fig. 2A). The transwell assays revealed that the overexpression of miR-877 inhibited the migration and invasion of Saos-2 and U2OS cells. In contrast, after miR-877 silenced, the opposite effects occurred (Fig. 2B and C). These results indicated that miR-877 could suppress the migration, invasion and proliferation of OS cells.

**Fig. 2** The effects of miR-877 on the clone formation, invasion and migration of OS cells. U2OS and Saos-2 cells were transfected with miR-877 mimic or miR-877 inhibitor. The clone formation (A), migration (B), and invasion (C) of OS cells were detected. *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC; *p < 0.05, **p < 0.01, ***p < 0.001 vs. NC-In.
miR-877 targeted GGCT

Next, we investigated whether miR-877 could target GGCT and regulate its expression. The mRNA and protein levels of GGCT were elevated in Saos-2 and U2OS cells (Fig. 3A and B). In addition, the mRNA level of GGCT in Saos-2 and U2OS cells was decreased, which was caused by the overexpression of miR-877 (Fig. 3C). The binding site of miR-877 on GGCT 3'-UTR was shown in Fig. 3D. The luciferase reporter assay revealed that the overexpression of miR-877 reduced the luciferase activity of WT GGCT 3'-UTR, while had no impact on the luciferase activity of Mut GGCT 3'-UTR (Fig. 3E). Furthermore, miR-877 overexpression decreased the protein level of GGCT in Saos-2 and U2OS cells (Fig. 3F). These data suggested that miR-877 targets GGCT and represses its expression.

GGCT mediated miR-877 on the migration, invasion, and proliferation of OS cells

To further determine whether GGCT mediates miR-877 in OS, the migration, invasion and proliferation of Saos-2 and U2OS cells which were co-overexpressed or co-silenced miR-877 and GGCT were detected. The transfection efficiencies of miR-877 mimic/inhibitor, sh-GGCT, and pcGGCT were shown in Fig. 4A and B. miR-877 overexpression inhibited the migration and invasion of Saos-2 and U2OS cells, and the overexpression of GGCT reversed this effects. The knockdown of miR-877 promoted the migration and invasion of Saos-2 and U2OS cells, and the silence of GGCT abolished this effects (Fig. 4C and D). The MTT assay showed that miR-877 overexpression suppressed the proliferation of Saos-2 and U2OS cells, while the forced expression of GGCT canceled the impacts. Besides, the knockdown of miR-877 facilitated the proliferation of Saos-2 and U2OS cells, and the effects were abrogated by GGCT silence (Fig. 4E and F). These results demonstrated that GGCT mediated miR-877 on the migration, invasion and proliferation of OS cells.

Discussions

In the current study, we found that miR-877 was down-regulated in OS tissues and cell lines. The overexpression of miR-877 inhibited the migration, invasion, and proliferation of OS cells, while its knockdown resulted in the opposite effects. Our further study clarified that miR-877 targeted GGCT and inhibited its expression. Mechanically, miR-877 suppressed the migration, invasion and proliferation of OS cells via targeting GGCT.

A large of studies reveal that miRNAs play crucial roles in OS. miR-708 is down-regulated in OS cell lines, and its overexpression can attenuate the proliferation and

Fig. 3 miR-877 targeted GGCT. (A) The mRNA level of GGCT in hFOB, U2OS and Saos-2 cells were detected by qRT-PCR. * $p < 0.05$, ** $p < 0.01$ vs. hFOB. (B) The protein levels of GGCT in U2OS and Saos-2 cells were detected by Western blot. (C) U2OS and Saos-2 cells were transfected with miR-877 mimic, and the mRNA level of GGCT was detected by qRT-PCR. *** $p < 0.001$ vs. NC. (D) The binding site of miR-877 on the 3'-UTR of GGCT. (E) The wild type (WT) and mutated (Mut) sequences of GGCT 3'-UTR were cloned into plasmid vector expressing luciferase. Each of recombinant vector was co-transfected into HEK-293T cells with miR-877 mimic. Then the luciferase activity was detected. ** $p < 0.01$ vs. NC. (F) U2OS and Saos-2 cells were transfected with miR-877 mimic, and the protein level of GGCT was detected by Western blot. NC, negative control.
contribute to the apoptosis of OS cells [18]. The expression of miR-627-3p is associated with the TNM classification in OS, which is down-regulated in OS tissues and cell lines. Besides, its overexpression can inhibit the migration, invasion and proliferation of OS cells [22]. miR-877 is a member of miRNAs and has been reported to exert anti-cancer functions in many types of cancers. In non-small cell lung cancer (NSCLC), miR-877 is down-regulated, and the recovery of its expression inhibits the proliferation and invasion of NSCLC cells in vitro [23]. In colorectal cancer, the overexpression of miR-877 attenuates the migration, invasion and proliferation of cancer cells, and also inhibits the tumor growth in vivo [24]. In hepatocellular carcinoma, miR-877 overexpression can partially enhance the paclitaxel sensitivity and repress the proliferation of cancer cells [25]. Neverthe-

Fig. 4 GGCT mediated the effects of miR-877 on the proliferation, migration and invasion of OS cells. U2OS and Saos-2 cells were transfected with miR-877 mimic, miR-877 mimic + pcGGCT, miR-877 inhibitor (miR-877-In), or miR-877 inhibitor + sh-GGCT. (A–B) The expression of GGCT was detected by qRT-PCR. (C–D) The migration and invasion of cells were detected by transwell assay. (E and F) The proliferation of cells was detected by MTT assay.
less, the specific effect of miR-877 in OS has not been determined. In this study, our data mining analyses of NCBI GEO public databases revealed that the level of miR-877 was reduced in OS. Our further results of qRT-PCR assay verified that miR-877 was down-regulated both in OS tissues and cell lines. Furthermore, our results revealed that the overexpression of miR-877 suppressed the migration, invasion and proliferation of OS cells, while the knockdown of miR-877 caused the opposite effects. Therefore, the present results demonstrated that miR-877 inhibited the migration, invasion and proliferation of OS cells.

Increasing evidence shows that miRNAs exert their functions in OS by targeting the 3'-UTR of mRNA and then inhibiting their expressions. For instance, miR-20a inhibits the tumor growth and proliferation of OS cells via targeting TAK1 and downregulating its expression [26]; miR-1284 represses the migration, proliferation and epithelial-mesenchymal transition of OS by targeting HMGB1 and inhibiting its expression [19]. We found that miR-877 targeted GGCT and suppressed its expression in OS cells in the present study. As previous studies reportedly, GGCT can act as an oncogene in many cancers, including glioma, prostate cancer, esophageal squamous carcinoma, breast cancer, gastric cancer, and ovarian cancer [9-13, 27]. However, there is no evidence to support the role of GGCT in oncogene in cancer, and its specific function remains unclear. Apart from these cancers, GGCT also can promote the progression of OS by facilitating the migration, invasion and proliferation of OS cells [14]. According to this researches, we speculated that miR-877 might exert its function in OS by targeting GGCT. As expected, our further results showed that GGCT was down-regulated in OS cell lines and mediated miR-877 on the migration, invasion and proliferation of OS cells. Hence, these results indicated that miR-877 exerted its function in OS by targeting GGCT and inhibiting its expression.

In conclusion, our findings suggested that miR-877 inhibited the migration, invasion, and proliferation of OS cells via targeting GGCT.

**Funding**

This study was supported by the funding of the Hebei provincial science and technology plan in 2018 (18277764D).

**Conflict of Interest**

All authors declare that they have no conflicts of interest in this work.

---

**Supplementary Fig. 1** MTT experimental results of miR-633 and miR-574-5p in OS cell lines.
References

1. Wang Z, Liu Z, Wu S (2017) Long non-coding RNA CTA sensitizes osteosarcoma cells to doxorubicin through inhibition of autophagy. Oncotarget 8: 31465–31477.
2. Marko TA, Diessner BJ, Spector LG (2016) Prevalence of metastasis at diagnosis of osteosarcoma: an international comparison. Pediatr Blood Cancer 63: 1006–1011.
3. Tang J, Shen L, Yang Q, Zhang C (2014) Overexpression of metadherin mediates metastasis of osteosarcoma by regulating epithelial-mesenchymal transition. Cell Prolif 47: 427–434.
4. Dai H, Lv YF, Yan GN, Meng G, Zhang X, et al. (2016) RanBP9/TSSC3 complex cooperates to suppress anoikis resistance and metastasis via inhibiting Src-mediated Akt signaling in osteosarcoma. Cell Death Dis 7: e2572.
5. Sasaki R, Osaki M, Okada F (2019) MicroRNA-based diagnosis and treatment of metastatic human osteosarcoma. Cancers (Basel) 11: 553.
6. Oakley AJ, Yamada T, Liu D, Coggan M, Clark AG, et al. (2008) The identification and structural characterization of C7orf24 as gamma-glutamyl cyclotransferase. An essential enzyme in the gamma-glutamyl cycle. J Biol Chem 283: 22031–22042.
7. Azumi K, Ikeda Y, Takeuchi T, Nomura T, Sabau SV, et al. (2009) Localization and characterization of gamma-glutamyl cyclotransferase in cancer cells. Mol Med Rep 2: 385–391.
8. Kageyama S, Hanada E, Li H, Tomita K, Yoshiki T, et al. (2015) Gamma-glutamylcyclotransferase: a novel target molecule for cancer diagnosis and treatment. Biomed Res Int 2015: 345219.
9. Zhang C, Li HR, Fan JB, Wang-Rodriguez J, Downs T, et al. (2006) Profiling alternatively spliced mRNA isoforms for prostate cancer classification. BMC Bioinformatics 7: 202.
10. Takemura K, Kawachi H, Eishi Y, Kitagaki K, Negi M, et al. (2014) Gamma-Glutamylcyclotransferase as a novel immunohistochemical biomarker for the malignancy of esophageal squamous tumors. Hum Pathol 45: 331–341.
11. Ran R, Liu Y, Gao H, Kuang Q, Zhang Q, et al. (2015) PEGylated hyaluronic acid-modified liposomal delivery system with anti-γ-glutamylcyclotransferase siRNA for drug-resistant MCF-7 breast cancer therapy. J Pharm Sci 104: 476–484.
12. Zhang W, Chen L, Xiang H, Hu C, Shi W, et al. (2016) Knockdown of GGCT inhibits cell proliferation and induces late apoptosis in human gastric cancer. BMC Biochem 17: 19.
13. Kageyama S, Li H, Taniguchi K, Kubota S, Yoshida T, et al. (2018) Mechanisms of tumor growth inhibition by depletion of gamma-glutamylcyclotransferase (GGCT): a novel molecular target for anticancer therapy. Int J Mol Sci 19: 2054.
14. Uejima D, Nishijo K, Kajita Y, Ishibe T, Aoyama T, et al. (2011) Involvement of cancer biomarker C7orf24 in the growth of human osteosarcoma. Anticancer Res 31: 1297–1305.
15. Bryan K, Terrile M, Bray IM, Domingo-Fernández R, Watters KM, et al. (2014) Discovery and visualization of miRNA-mRNA functional modules within integrated data using bicluster analysis. Nucleic Acids Res 42: e17.
16. Hui Y, Huang Y, Ding X, Wang L (2019) MicroRNA-152 suppresses cell proliferation and tumor growth of bladder cancer by targeting KLF5 and MKK7. Aging Pathobiol Ther 1: 10–16.
17. Ge B, Wu H, Shao D, Li S, Li F (2019) Interfering with miR-24 alleviates rotenone-induced dopaminergic neuron injury via enhancing autophagy by upregulating DJ-1. Aging Pathobiol Ther 1: 17–24.
18. Chen G, Zhou H (2018) MiRNA-708/CUL4B axis contributes into cell proliferation and apoptosis of osteosarcoma. Eur Rev Med Pharmacol Sci 22: 5452–5459.
19. Lv S, Guan M (2018) miRNA-1284, a regulator of HMGB1, inhibits cell proliferation and migration in osteosarcoma. Biosci Rep 38: BSR20171675.
20. Meng Y, Gao R, Ma J, Zhao J, Xu E, et al. (2017) MicroRNA-140-5p regulates osteosarcoma chemoresistance by targeting HMGN5 and autophagy. Sci Rep 7: 416.
21. Sun Y, Cao L, Lin JT, Yuan Y, Cao ZL, et al. (2019) Upregulated miRNA-1236-3p in osteosarcoma inhibits cell proliferation and induces apoptosis via targeting KLF8. Eur Rev Med Pharmacol Sci 23: 6053–6061.
22. He M, Shen P, Qiu C, Wang J (2019) miR-627-3p inhibits osteosarcoma cell proliferation and metastasis by targeting PTN. Aging (Albany NY) 11: 5744–5756.
23. Zhou G, Xie J, Gao Z, Yao W (2019) MicroRNA-877 inhibits cell proliferation and invasion in non-small cell lung cancer by directly targeting IGF-1R. Exp Ther Med 18: 1449–1457.
24. Meng F, Ou J, Liu J, Li X, Meng Y, et al. (2019) MicroRNA-877 is downregulated in cervical cancer and directly targets MACC1 to inhibit cell proliferation and invasion. Exp Ther Med 18: 3650–3658.
25. Huang X, Qin J, Lu S (2015) Up-regulation of miR-877 induced by paclitaxel inhibits hepatocellular carcinoma cell proliferation though targeting FOXM1. Int J Clin Exp Pathol 8: 1515–1524.
26. Yuan G, Zhao Y, Wu D, Gao C, Jiao Z (2018) microRNA-20a upregulates TAK1 and increases proliferation in osteosarcoma cells. Future Oncol 14: 461–469.
27. Shen SH, Yu N, Liu XY, Tan GW, Wang ZX (2016) Gamma-glutamylcyclotransferase promotes the growth of human glioma cells by activating Notch-Akt signaling. Biochem Biophys Res Commun 471: 616–620.