

## ORIGINAL ARTICLE

# Biological functions of miRNA-188-5p/XRCC5 in glioma

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## Summary

**Purpose:** The purpose of this study was to uncover the influence of microRNA-188-5p (miRNA-188-5p) on the malignant progression of glioma, thus providing a new direction in the early diagnosis and prediction of disease progression.

**Methods:** MiRNA-188-5p levels in 44 glioma tissues and paracancerous ones were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Its influence on pathological indicators and prognosis in glioma patients was analyzed. In glioma cell lines, regulatory effects of miRNA-188-5p on cell phenotypes were examined by cell counting kit-8 (CCK-8), wound healing and Transwell assay, respectively. Moreover, the interaction between miRNA-188-5p and XRCC5, as well as their involvement in the development of glioma were finally illustrated.

**Results:** MiRNA-188-5p was downregulated in glioma

samples. Glioma patients expressing a low level of miRNA-188-5p had a worse prognosis. Overexpression of miRNA-188-5p remarkably attenuated proliferative and migratory abilities of glioma cells. XRCC5 was the downstream gene of miRNA-188-5p, and its level was negatively regulated by miRNA-188-5p. Besides, XRCC5 was upregulated in glioma samples. Moreover, XRCC5 was responsible for the inhibitory effects of miRNA-188-5p on the malignant progression of glioma.

**Conclusions:** MiRNA-188-5p is linked to malignant progression in glioma patients, and it inhibits the proliferative and migratory abilities of glioma cells by binding XRCC5 and then negatively regulating its level.

**Key words:** MiRNA-188-5p, XRCC5, glioma, malignant progression

## Introduction

Glioma is a frequent primary tumor in the central nervous system, accounting for 40% of intracranial tumors. It is featured with fast course, easy relapse, strong proliferative and invasive growth [1-3]. Histologically, astrocytoma is the most frequent subtype of glioma, followed by glioblastoma, ependymoma and medulloblastoma. Oligodendroglioma, mixed glioma and pinealoma are rarely seen in clinical practice [4,5]. Based on the biological behaviors of glioma, the WHO classifies glioma

into stage I-IV. Each stage of glioma also includes multiple pathological subtypes [5]. The anatomic location of glioma is relatively hidden and it easily invades adjacent tissues. As a result, glioma is difficult to be completely removed by surgery. In addition, radiotherapy/chemotherapy resistance rate is high in glioma patients. The average survival of glioma is only 12 months [6,7]. In China, the incidence of glioma has been on the rise and it is urgent to develop novel therapeutic strategies for

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this disease [8,9]. Non-invasive molecular markers for glioma have been well addressed in recent studies [10,11].

Glioma is a multiple-factor, multi-step, progressive disease. Its development involves imbalanced oncogenes and tumor suppressors, as well as abnormally activated/inactivated pathways [5,12]. MicroRNAs (miRNAs) are vital regulators involved in tumor development [13,14]. They are non-coding, single-stranded RNAs with a phosphate group at the 5' end and a hydroxyl group at the 3' end. By recognizing and binding to mRNAs 3' untranslated region (3'UTR), miRNAs exert post-transcriptional regulations via degrading them or inhibiting their translation [14,15]. MiRNA-188-5p is a cancer-associated miRNA, which is highly conserved in humans, rats, mice and other vertebrates [16,17]. It is reported that miRNA-188-5p is a new hallmark for retinoblastoma, which is downregulated in retinoblastoma samples and inhibits the proliferation and metastasis [18]. The biological functions of miRNA-188-5p in glioma are yet unclear.

Bioinformatics analysis uncovered the interaction between miRNA-188-5p and XRCC5. XRCC5 locates on human chromosome 2q33-35. XRCC5-encoded Ku80 and XRCC6-encoded Ku70 constitute a heterodimer, that is, Ku protein. As a conserved DNA adhesion protein, Ku protein participates in V(D)J recombination and double-strand break repair. It promotes double-strand reconnection and recombination. Previous experimental evidence has demonstrated that Ku protein exerts an important role in maintaining the functional integrity of the genome and repairing the damage caused by carcinogenic factors [19,20]. Abnormal expression and disordered activity of XRCC5 display a certain impact on tumor development [21]. This study aimed to uncover the key roles of miRNA-188-5p in the development of glioma and the involvement of XRCC5.

## Methods

### *Patients and glioma samples*

Glioma tissues and adjacent normal ones were collected from 44 glioma patients. Tissues were pathologically diagnosed and prepared for the following experiments. Clinical data and follow-up information were recorded. This study got approval by the Ethics Committee of Jiaozhou Central Hospital of Qingdao and it was conducted after informed consent was obtained from each subject.

### *Cell lines and reagents*

The human glioma cell lines U251, U87, T98-G, A172s and a human brain normal glial cell line (HEB) were purchased from American Type Culture Collec-

tion (ATCC) (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) in a 5% CO<sub>2</sub> incubator at 37°C. 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 µg/mL streptomycin were added in the culture medium.

### *Transfection*

Cells were inoculated in 6-well plates with 5×10<sup>4</sup> cells/well. After cell growth to 50-70% confluence, they were transfected with plasmids constructed by GenePharma (Shanghai, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Transfection efficacy was tested by quantitative real-time polymerase chain reaction (qRT-PCR).

### *Cell counting kit-8 (CCK-8) assay*

Cells were inoculated in 96-well plates with 2×10<sup>5</sup> cells per well. At the appointed time points, absorbance value at 490 nm of each sample was recorded using the CCK-8 kit (RIBOBIO, Guangzhou, China) for plotting the viability curves.

### *Wound healing assay*

Cells were inoculated in 6-well plates and grew to 90% confluence. After creation of an artificial wound in a cell monolayer, medium with 1% FBS was replaced. 24 hours later, wound closure was captured for calculating the percentage of wound healing.

### *Transwell migration assay*

Transwell chambers (Millipore, Billerica, MA, USA) were inserted in each well of a 24-well plate. 200 µL of suspension (1×10<sup>5</sup> cells/mL) was applied in the upper layer of a chamber with 700 µL of medium containing 20% FBS in the bottom. After 48-h incubation, bottom cells reacted with 15 min methanol, 20 min crystal violet and captured using a microscope. Finally, migratory cells were counted in 10 random selected fields per sample.

### *QRT-PCR*

Extracted RNAs by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) were purified by DNase I treatment, and reversely transcribed into complementary deoxyribose nucleic acids (cDNAs) using Primescript RT Reagent (TaKaRa, Otsu, Japan). The obtained cDNAs underwent qRT-PCR using SYBR®Premix Ex Taq™ (TaKaRa, Otsu, Japan). Each sample was performed in triplicate. Relative level was calculated by 2<sup>-ΔΔCt</sup> and normalized to that of β-actin or U6. MiRNA-188-5p: Forward: 5'-CCCTCTCTCACATCCCTTGCAT-3', Reverse: 5'-ATCCTGCAAACCTGCATGTG-3'; U6: Forward: 5'-CGCAAGGATGACACGCAAATTC-3', Reverse: 5'-TATATCACTCTTGCTTCA-3'; XRCC5: Forward: 5'-GGGGAGCACAATTTCCCTTC-3', Reverse: 5'-GGAAGTGAAGTCAAGGCAAG-3'; β-actin: Forward: 5'-CCTGGCACCCAGCACAAT-3', Reverse: 5'-GCTGATCCACATCTGCTGGAA-3'.

### *Western blot*

Cells were lysed for isolating proteins and electrophoresed. Protein samples were loaded on polyvi-

nylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, non-specific antigens were blocked in 5% skim milk for 2 h. Primary and secondary antibodies were applied for indicated time. Band exposure and grey value analyses were finally conducted.

Luciferase assay

Wild-type and mutant-type XRCC5 vectors were constructed based on predicted consequential pairing of XRCC5 3'UTR and miRNA-188-5p. HEK293 cells were pre-seeded in a 24-well plate. The other day, they were co-transfected with NC mimic/miRNA-188-5p mimic and XRCC5-WT/XRCC5-MUT. After 48 h cell culture, they were lysed for measuring luciferase activity (Promega, Madison, WI, USA).

Statistics

SPSS 22.0 statistical package (IBM, Armonk, NY, USA) was used for data analyses. Data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed by the t-test. The relationship between miRNA-188-5p level and pathological indicators of glioma patients was evaluated by Chi-square test. Pearson's correlation test was applied for evaluating the relationship between two genes. Kaplan-Meier curves and log-rank test were used for survival analysis. P<0.05 was considered as statistically significant.

Results

Downregulated miRNA-188-5p in glioma tissues and cell lines

QRT-PCR data showed a lower level of miRNA-188-5p in glioma tissues than adjacent normal ones (Figure 1A). Using the clinical data of 44 involved glioma patients, we analyzed the relationship between miRNA-188-5p level and age, gender, T stage. As shown in Table 1, miRNA-188-5p level was not correlated with the above indicators in glioma patients. As expected, it was also downregulated in glioma cell lines (Figure 1B). Follow-up information of each included patient was collected. Kaplan-Meier and log-rank test revealed a poor prognosis in glioma patients expressing a low level of miRNA-188-5p (p<0.01).

Overexpression of miRNA-188-5p inhibited proliferative and migratory abilities in glioma

We constructed miRNA-188-5p overexpression model by transfection of miRNA-188-5p mimic in T98-G and U87 cells (Figure 2A). In glioma cells overexpressing miRNA-188-5p, cell viability was markedly decreased from 24 h to

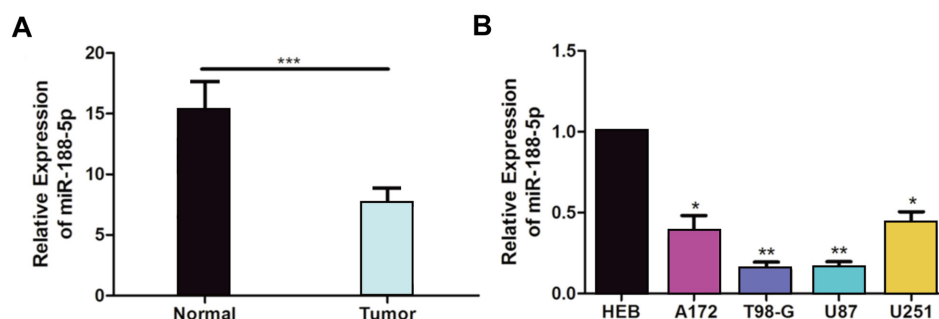


Figure 1. Downregulated miR-188-5p in glioma tissues and cell lines. A: MiR-188-5p levels in glioma tissues and adjacent normal ones. B: MiR-188-5p levels in glioma cell lines. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

Table 1. Association of miR-188-5p expression with clinicopathologic characteristics of glioma

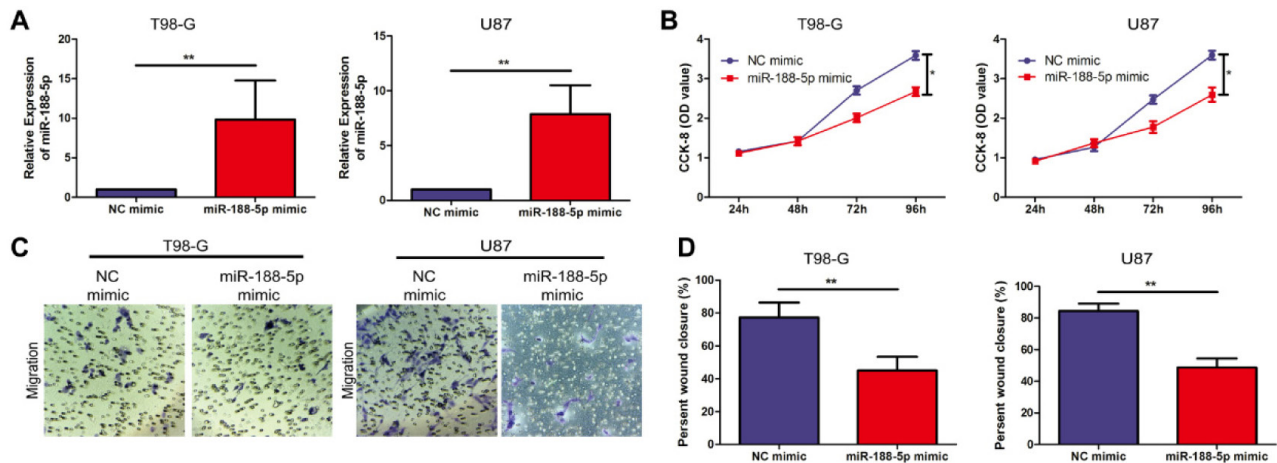
Parameters	n	miR-188-5p expression		p
		High (n=23)	Low (n=21)	
Age (years old)				0.564
<55	23	13	10	
≥55	21	10	11	
Gender				0.555
Male	21	10	11	
Female	23	13	10	
T stage				0.944
T1-T2	27	14	13	
T3-T4	17	9	8	

96 h than those of controls (Figure 2B). Besides, both migratory cell number and wound closure percentage were reduced after overexpression of miRNA-188-5p in T98-G and U87 cells, suggesting the attenuated migratory ability (Figures 2C, 2D).

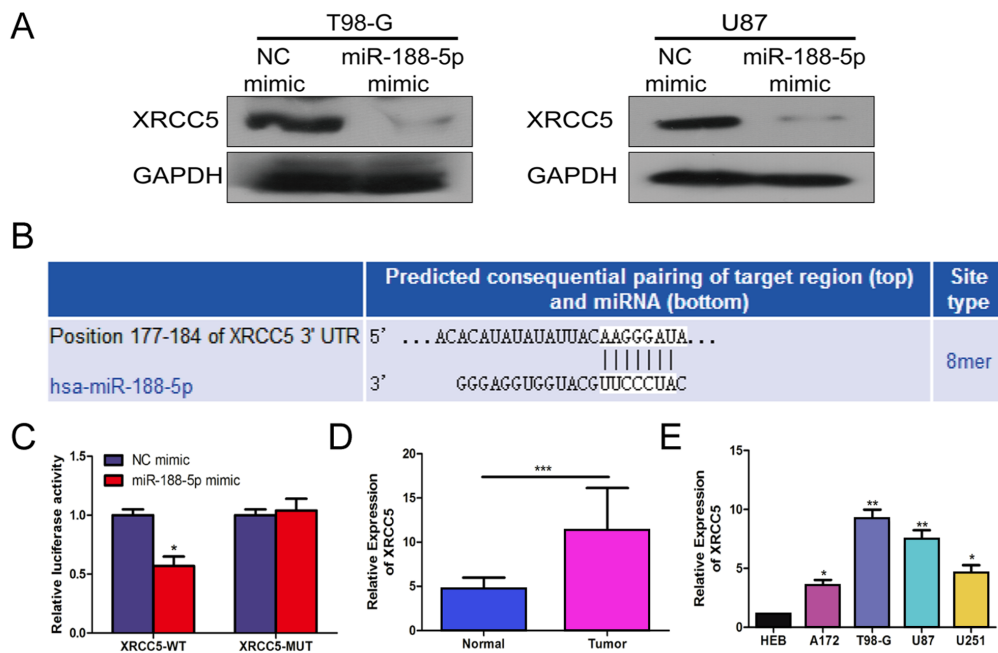
*XRCC5* was the downstream gene of *miRNA-188-5p*

Protein level of *XRCC5* was markedly down-regulated in T98-G and U87 cells overexpressing

*miRNA-188-5p* (Figure 3A). A potential interaction between *miRNA-188-5p* and *XRCC5* was predicted by bioinformatics analysis (Figure 3B). Overexpression of *miRNA-188-5p* greatly decreased luciferase activity in wild-type *XRCC5* vector, which was unchangeable in mutant-type, confirming that *XRCC5* was the downstream gene binding to *miRNA-188-5p* (Figure 3C). QRT-PCR data yielded that *XRCC5* was upregulated in glioma tissues and cell lines (Figures 3D, 3E).



**Figure 2.** Overexpression of *miR-188-5p* inhibited proliferative and migratory abilities in glioma. **A:** Transfection efficacy of *miR-188-5p* mimic in T98-G and U87 cells. **B:** Viability in T98-G and U87 cells transfected with NC mimic or *miRNA-188-5p* mimic. **C:** Migration in T98-G and U87 cells transfected with NC mimic or *miR-188-5p* mimic. **D:** Wound closure percentage in T98-G and U87 cells transfected with NC mimic or *miR-188-5p* mimic. \* $p < 0.05$ , \*\* $p < 0.01$ .



**Figure 3.** *XRCC5* was the downstream gene of *miRNA-188-5p*. **A:** Protein level of *XRCC5* in T98-G and U87 cells transfected with NC mimic or *miR-188-5p* mimic. **B:** Predicted consequential pairing of *XRCC5* 3'UTR and *miR-188-5p*. **C:** Luciferase activity in HEK293 cells co-transfected with NC mimic/*miRNA-188-5p* mimic and *XRCC5*-WT/*XRCC5*-MUT. **D:** *XRCC5* levels in glioma tissues and adjacent normal ones. **E:** *XRCC5* levels in glioma cell lines. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

*XRCC5 abolished regulatory effects of miRNA-188-5p on glioma cell phenotypes*

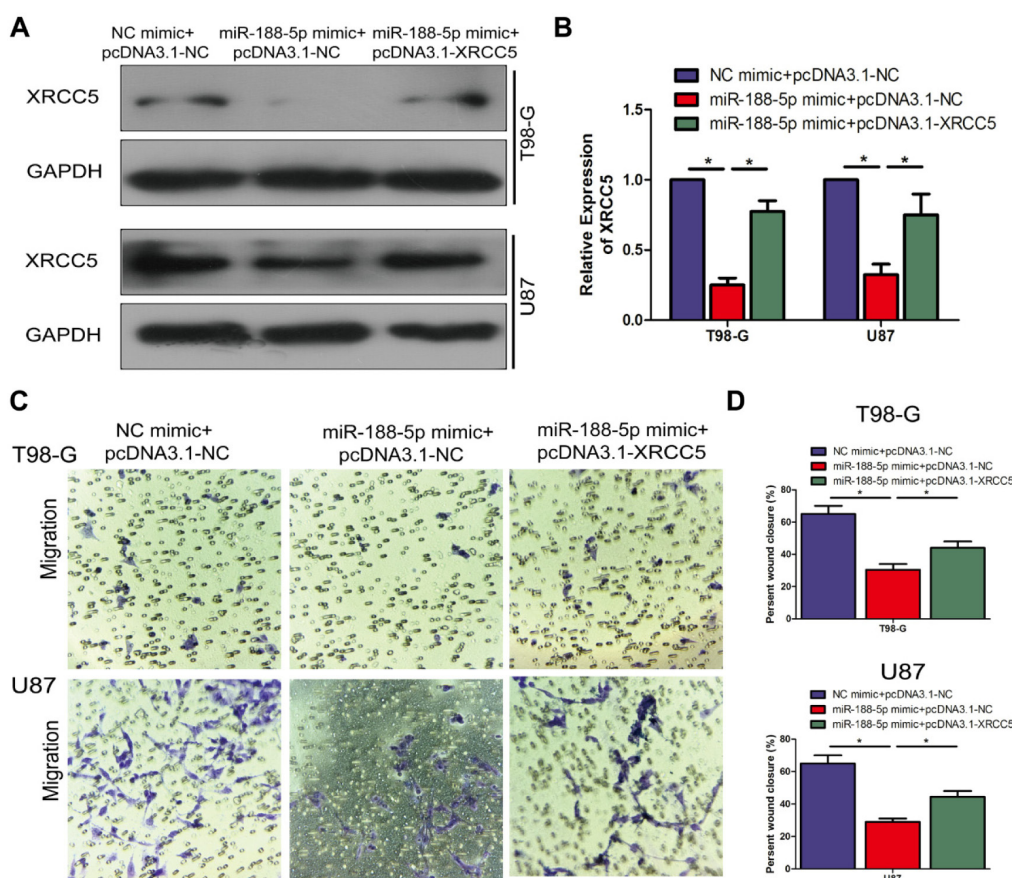
To uncover how miRNA-188-5p and XRCC5 synergistically regulated the development of glioma, they were co-overexpressed in glioma cells. Higher protein and mRNA levels of XRCC5 were seen in glioma cells co-overexpressing miRNA-188-5p and XRCC5 than those overexpressing miRNA-188-5p (Figures 4A, 4B). The inhibitory effects of overexpressed miRNA-188-5p on migratory ability and wound closure were abolished by co-overexpression of miRNA-188-5p and XRCC5 (Figures 4C, 4D).

**Discussion**

Glioma is a malignant tumor originating from neuroectoderm. It is a common and aggressive intracranial malignancy, accounting for 50% of adult intracranial malignancies. The mortality of glioma is extremely high, especially in high-grade gliomas [1-3]. Generally speaking, the pathogenesis of glioma involves both host factors and environmental factors [4,5]. So far, surgical resection combined

with postoperative radiotherapy/chemotherapy is preferred to glioma. With the development of microneurosurgery, the resection rate of gliomas has remarkably increased. Nevertheless, the postoperative recurrence rate is significantly high even after active treatment [6,9]. In addition, glioma is featured with active infiltration and invasion of the surrounding normal brain tissues, as well as abundant blood supply in tumor lesions, leading to difficulties in complete resection and poor prognosis [6-8]. Molecule targeted therapies for tumors developed from single-target therapies into multiple-target therapies, which not only focuses on multiple targets but also the interacted pathways [9-12].

MiRNAs have 20 nucleotides in length and they are not easily degraded compared to mRNAs [13-15]. Previous studies have demonstrated the possibility of miRNAs as diagnostic markers by detecting their differential expressions in paraffin-embedded tissue samples [14]. The specific expression profiles of miRNAs in tumor tissues also indicate their diagnostic potentials [16,17]. So far, there are hundreds of miRNAs discovered to be linked to



**Figure 4.** XRCC5 abolished the regulatory effects of miRNA-188-5p on glioma cell phenotypes. T98-G and U87 cells were co-transfected with NC mimic+pcDNA3.1-NC, miR-188-5p mimic+pcDNA3.1-NC or miR-188-5p mimic+pcDNA3.1-XRCC5. **A:** Protein level of XRCC5. **B:** The mRNA level of XRCC5. **C:** Migratory cell number. **D:** Wound closure percentage. \*p<0.05.

glioma. MiRNA-21 is downregulated in glioma tissues, which regulates glioma cell growth and invasiveness [22]. In the present study, miRNA-188-5p was downregulated in glioma tissues we collected, which was closely related to distant metastasis rate in glioma patients and is suggested that miRNA-188-5p may exert an anti-cancer role in glioma. Subsequently, we constructed miRNA-188-5p overexpression model in T98-G and U87 cells by transfection of miRNA-188-5p mimic. Overexpression of miRNA-188-5p markedly decreased viability, migratory cell number and wound closure percentage in glioma, suggesting its vital regulations on glioma cell phenotypes.

Potential downstream targets of a miRNA and the corresponding seed sequences can be predicted online [23,24]. A single miRNA may have hundreds of downstream targets. The most possible downstream target is selected by cross-over studies in several websites and then functional verification [24]. Here, a potential interaction between miRNA-188-5p and XRCC5 was predicted. Subsequent luciferase assay confirmed their binding relationship that miRNA-188-5p could post-transcriptionally

regulate XRCC5 level. Converse to miRNA-188-5p, XRCC5 was highly expressed in glioma samples. Transfection of miRNA-188-5p mimic downregulated mRNA and protein levels of XRCC5, displaying a negative correlation between each other. Finally, rescue experiments revealed that overexpression of XRCC5 abolished the inhibitory effects of overexpressed miRNA-188-5p on proliferative and migratory abilities in glioma cells. To sum up, miRNA-188-5p inhibited the malignant development of glioma through a negative feedback loop involving XRCC5.

## Conclusions

MiRNA-188-5p is linked to the malignant progression in glioma patients. It inhibits the proliferative and migratory abilities in glioma cells by binding to XRCC5 and then negatively regulating its level.

## Conflict of interests

The authors declare no conflict of interests.

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