

The effect of miR-23b-3p on growth hormone in pituitary cells of Yanbian yellow cattle

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Research

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Abstract

Background There is a relationship between miR-23b-3p and GH in pituitary of Yanbian yellow cattle. However, the specific mechanism of the effect of miR-23b-3p on GH in pituitary of Yanbian yellow cattle is still unclear. This study aimed to evaluate the effect of miR-23b-3p on growth hormone (GH) in pituitary cells of Yanbian yellow cattle.

Methods The primary culture of Yanbian yellow cattle pituitary cells was carried out, and mimics (miR-23b-3p-mi group), mimics reference substance (NC group), inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p were transfected into the established pituitary primary cells. After 48 h, the cells were collected and the total RNA and protein were extracted. The mRNA transcription and protein expression level of GH and miR-23b-3p target genes were detected by real time fluorescence quantitative PCR (qPCR) and Western blot, respectively. The target relationship of miR-23b-3p was validated by double luciferase reporter gene system.

Results Compared with the NC control group, GH mRNA transcription and protein expression level in pituitary cells of Yanbian yellow cattle was significantly decreased by adding miR-23b-3p mimics ($P < 0.01$), while compared with the iNC control group, GH mRNA transcription and protein expression level were significantly increased by adding miR-23b-3p inhibitor ($P < 0.05$). The result of bioinformatics analysis and double luciferase reporter gene system validation proved that miR-23b-3p targeted 3'UTR of pituitary specific transcription factor 1 (POU1F1). Compared with the NC control group, POU1F1 mRNA transcription and protein expression level were significantly inhibited by the addition of miR-23b-3p mimics ($P < 0.01$), while compared with the iNC control group, POU1F1 mRNA transcription and protein expression level were significantly increased by the addition of miR-23b-3p inhibitor ($P < 0.01$).

Conclusions miR-23b-3p could regulate GH in pituitary cells by regulating POU1F1 gene.

Background

Yanbian yellow cattle are characterized by slow growth rate, long growth cycle, and low market value, leading to prolonged feeding periods and wastage of feed resources, high feeding costs, and consequently negative effects on the Yanbian yellow cattle breeding industry[1–3]. Therefore, how to improve the growth rate of Yanbian cattle is the key to accelerate the rapid development of Yanbian cattle breeding industry.

MiRNAs are non-coding small RNAs that perform important functions in animals. They inhibit the expression of target genes after transcription by binding to the 3'UTR region of the target gene mRNA[4–7]. Some studies have shown that miRNAs in play an important role in regulating the growth and development of animals. For example, Melnik et al. found that miRNA in milk exosome could promote calf growth[8–10]. Chen et al. used miR-PC-86 and miR-PC-263 derived from pig exosome to treat C2C12 cells and found that miR-PC-86 and miR-PC-263 could regulate the expression of IGF-1R receptor on

C2C12 cells and further regulate the growth of porcine muscle cells[11]. So some miRNAs may related to animal growth and development.

The growth axis plays an essential role in the growth and development of animals[12–14]. GH secreted by the pituitary gland is a key component of the growth axis[15–17]. GH can be transported by binding to growth hormone binding protein (GHBP) and to growth hormone receptor (GHR) in target organs, which promotes the production of insulin-like growth factors (IGFs). IGFs are then transported to whole body tissues and cells by binding to insulin-like growth factor binding protein (IGFBP) to stimulate the growth and differentiation of bone and chondrocyte and regulate the metabolism of protein, sugar and fat, etc[18–22]. Some studies showed that there was a relationship between miRNAs and GH[23].

In our previous research, we selected Yanbian yellow cattle to analyze the effects of miRNAs on the growth of Yanbian yellow cattle using bioinformatics approach. Finally the differentially expressed miR-23b-3p was selected out[24]. However, the specific mechanism of the effect of miR-23b-3p on GH in pituitary of Yanbian yellow cattle is still unclear. Therefore, in this study, the regulation mechanism of miR-23b-3p on GH in pituitary of Yanbian yellow cattle was evaluated at the level of pituitary cells in vitro. This study will provide a theoretical basis for further studying on the regulation mechanism of miRNAs on growth and development of animals.

Materials And Methods

Primary culture of pituitary cells of Yanbian yellow cattle

The pituitary tissues of three Yanbian yellow cattle were separated under aseptic conditions and cut into 1 mm × 1 mm tissue mass, respectively and then were washed with PBS for three times at pH 7.0. The tissue precipitation was transferred to culture flask, and 0.5 mL 0.25% collagenase and trypsin (TaKaRa, Tokyo, Japan) were added to each pituitary tissues and incubated in shaking bed at 37 °C for 25 min. The pituitary cells were filtered with 100 meshes cell sieve. The filtrate was centrifuged at 4 °C, 2 000 rpm for 10 min and the supernatant was discarded. Cells were suspended in DMEM/F12 medium and cultured in 75 mL culture flask at 37 °C and 5% CO₂ incubator. When the cells grew up to 80% of convergence, they were digested with 0.25% collagenase. The cells were isolated by differential adherence method and primary cultured pituitary cells were obtained.

Expression level of miR-23b-3p in pituitary cells

After the primary culture of Yanbian yellow cattle pituitary cells was established, the pituitary cells were inoculated into 6-well culture plate according to the density of 2.0×10^6 cells/hole, and transfection test was carried out when the cell confluence was 60%-70%. The pituitary cells were washed with 2 mL PBS once before transfection, and 0.8 mL DMEM/F12 medium was added. Mimics (miR-23b-3p-mi group), mimics reference substance (NC group), inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p were diluted to 100 μ L DMEM/F12 medium respectively. The miR-23b-3p

mimic, inhibitor, NC and inhibitor were purchased from Sangon Biotech Co. (Shanghai, China). The sequences were as follows: miR-23b-3p mimic (5'-AUCACAUUGCCAG GGAUUACC-3'), miR-23b-3p inhibitor (5'-GGUAAUCCUGGCAAUGUGAU-3'), NC (5'-UCGCACGGCCAAAUCCACGU-3'), and iNC (5'-AGGUACGAAUCCGGAAGUA C-3'). The 10 μ L transfection reagent Lipofectamine™ 2000 (QIAGEN, Hilden, Germany) was diluted to 100 μ L DMEM/F12 culture medium and then mixed with above diluents in a ratio of 1:1, respectively. After incubation at room temperature for 10 min, 200 μ L mixture was added into the cell pore of the preconditioned cells, and the final concentration of mimics, mimics reference substances, inhibitor and inhibitor reference substances of miR-23b-3p was 200 pmol/L, with three replicates in each group. After culture for 48 h, the cells were collected and the total RNA was extracted. miR-23b-3p expression level was detected using fluorescence quantitative PCR (qPCR).

Total RNA was extracted from pituitary cells by Trizol reagent and digested with DNase I (TaKaRa, Tokyo, Japan) to remove trace DNA contamination. The cDNA synthesis was catalyzed by M-MLV reverse transcriptase (TaKaRa, Tokyo, Japan) using total RNA as a template and the specific neck loop primer (5'CTCAACTGGTGTCTGGAGTCGGCAATT AGTTGAGCACAAATT3') (Qi et al., 2015). Primer Premier 6.0 software was used to design the forward and reverse primers for the PCR amplification of miRNA and U6 genes. The forward and reverse primers of miR-23b-3p were 5'-ATCACATTTGTAGACACG-3' and 5'-ACCACTCGTGGAGAGC - 3', respectively; The forward and reverse primers of U6 were 5'-CTCGCTTCGGCAGCACACA-3' and AACGCTTCACGAATTTGCGT, respectively. PCR mixture contained 5 μ L of SYBR Green Master Mix (TaKaRa, Tokyo, Japan), 0.5 μ L of 10 mM each of primers, 1 μ L of cDNA and 3 μ L of PCR water. The PCR conditions were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 20 s and 72 °C for 20 s, and the fluorescence signals were then collected. The expression level of miR-1468 was calculated by the $2^{-\Delta\text{CT}}$ method. The formula is as follows: $\Delta\text{Ct} = \text{miR-23b-3p Ct} - \text{U6 Ct}$.

Effect of miR-23b-3p on GH mRNA transcription level in Yanbian yellow cattle pituitary cells

The cDNA synthesise was catalyzed by M-MLV reverse transcriptase (TaKaRa, Tokyo, Japan) using total RNA as template and OligodT-18 as primer. Primer Premier 6.0 software was used to design upstream and downstream primers of GH gene and β -actin gene in Yanbian yellow cattle. The upstream and downstream primers of GH were: CTCAA CTGCTGGCTGCCGACAGCTA and CGATGTCTGCTGGGCTCGTCC, respectively; The upstream and downstream primers of β -actin were: CCACGAACTACCTTCAACTC and CCCACGAACTA CCTTCAACTC, respectively. The PCR reaction system consisted of 10 μ L of SYBR Green Master Mix, 0.5 μ L of 10 mM each of primers, 1 μ L of cDNA and 8.2 μ L of sterile water. The PCR reaction procedure was: 95 °C for 1 min, then 35 cycles of 95 °C for 15 s, 56 °C for 15 s and 72 for 35 s, and the fluorescence signals were collected at the end of the extension. The expression level of GH gene was calculated by $2^{-\Delta\text{CT}}$ method. The formula was: $\Delta\text{Ct} = \text{GH Ct} - \beta\text{-actin Ct}$.

Effect of miR-23b-3p on GH protein expression in pituitary cells of Yanbian yellow cattle

According to the steps of the total protein extraction kit (Roche, Basel, Switzerland), the total protein was extracted from the above obtained cells, and the GH protein was detected by Western blot method. The specific steps were as follows: 10% PAGE separating gel and 5% PAGE concentrating gel were prepared according to the conventional method and put into the vertical electrophoresis tank. After solidification, the wood comb was pulled out and the 1 × glycine buffer was added into the comb hole, then 50 g prepared total protein sample was added into the comb hole for electrophoresis. After electrophoresis, the protein glue was transferred to PVDF (polyvinylidene fluoride) membrane (Roche, Basel, Switzerland) with a voltage of 110 V for 60–70 min. Then the PVDF membrane was taken out and stained with Ponceau red to test the effect of transmembrane. The PVDF membrane was washed for several times with 1 × TBST (Tris-HCl and tween buffer) to remove the Ponceau dye solution, and then sealed for 2 h at room temperature with 5% skimmed milk powder. After that, the sealed PVDF membrane was placed in a 5 mL centrifugal tube and 2 mL first antibody of GH protein was added into the centrifugal tube and incubated for 12 h at 4 C. After incubation, PVDF membrane was washed 3 times with 1 × TBST solution for 10 min each time. The washed PVDF membrane was placed in a new 5 mL centrifugal tube and incubated at room temperature for 2 h with 2 mL 1:5000 diluted second antibody of GH protein. After incubation, the PVDF membrane was washed 3 times with 1 × TBST for 5 min each time. Then the PVDF membrane was placed in the luminescent liquid for 1 min and exposed in the gel imaging system. The relative expression of GH protein was calculated by comparing with the expression of β -actin as a reference gene.

Target gene prediction of miR-23b-3p

Targetscan and RNA hybridization analysis software were used to predict the target relationship between miR-23b-3p and GH secretion related main genes including growth hormone releasing hormone receptor (GHRHR), somatostatin receptor 2 (SSTR2), lymphoid enhancer 1 (LEF1), pituitary specific transcription factor 1 (POU1F1), somatostatin receptor 5 (SSTR5) and cyclic adenosine phosphate effector binding protein 1 (CREB1).

Verification of the target relationship between miR-23b-3p and POU1F1

The upstream and downstream sequence within 30 bp of POU1F1 3'UTR region matched with the seed sequence of miR-23b-3p was synthesized, and the 5 bases of the seed sequence of miR-23b-3p were mutated artificially as a control. The synthetic fragments and mutant fragments were digested by XhoI and XbaI (TaKaRa, Tokyo, Japan). Then the recombinant pirGLO-POU1F1-3'UTR expression vector and mutant vector were constructed by linking the digested products with the luciferase gene report vector (pirGLO vector), respectively. Mimic (miR-23b-3p-mi group) and mimics reference substance (NC group) of miR-23b-3p and the normal or mutant vector of pirGLO-POU1F1-3'UTR were diluted to 200 pmol/L respectively, and then co-transfected into CHO cells by transfection reagent Lipofectamine™ 2000. The

specific transfection process was as described above. Cells were collected and lysed after incubating for 48 h and luciferase activity was detected by luciferase detection kit (Promega, Wisconsin, USA).

Effects of miR-23b-3p on the transcriptional level and protein expression of POU1F1 in pituitary cells of Yanbian yellow cattle

The detection of mRNA transcription level and protein expression of POU1F1 referred to the detection methods of mRNA transcription level and protein expression of GH mentioned above.

Statistical analyses

All statistical analyses were performed using SPSS 17.0 statistical software (SPSS 17.0, Chicago, IL, USA). A t-test was performed to examine the significant difference among treatments tested by one-way ANOVA. $P < 0.05$ represented significant difference, $P < 0.01$ represented extremely significant difference.

Results

Expression level of miR-23b-3p in pituitary cells

The pituitary cells were transfected with miR-23b-3p mimics and inhibitor, and qPCR was used to detect miR-23b-3p expression. Transfection of miR-23b-3p mimics significantly increased miR-23b-3p level, while transfection of miR-23b-3p inhibitor significantly decreased miR-23b-3p level ($P < 0.01$) (Fig. 1a and 1b).

Effect of miR-23b-3p on GH mRNA transcription level in pituitary cells of Yanbian yellow cattle

In order to analyze the effect of miR-23b-3p on pituitary GH mRNA transcription level in Yanbian yellow cattle, the primary cultured pituitary cells of Yanbian yellow cattle were transfected with mimics (miR-23b-3p-mi group), mimics reference substance (NC group), inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p, and then the transcriptional level of GH gene was detected by qPCR. The results showed that the GH mRNA transcription level in pituitary cells of Yanbian yellow cattle in miR-23b-3p group was extremely significantly lower than that in NC group ($P < 0.01$) (Fig. 2a), while the GH mRNA transcription level in pituitary cells of Yanbian yellow cattle in miR-23b-3p-in group was significantly higher than that in iNC group ($P < 0.05$) (Fig. 2b). These results suggested that miR-23b-3p could regulate GH transcription.

Effect of miR-23b-3p on GH protein expression level in pituitary cells of Yanbian yellow cattle

In order to further verify the effect of miR-23b-3p on pituitary GH expression in Yanbian yellow cattle, GH protein expression level was further detected by Western blot based on the transcription results of GH mRNA. The results showed that GH protein expression level in pituitary cells of Yanbian cattle in miR-23b-3p-mi group was extremely significantly lower than that in NC group ($P < 0.01$) (Fig. 3a), while GH protein expression level in pituitary cells of Yanbian yellow cattle in miR-23b-3p-in group was extremely significantly higher than that in iNC group ($P < 0.01$) (Fig. 3b). These results suggested that miR-23b-3p could regulate GH protein expression.

Prediction of the target relationship between miR-23b-3p and GH secretion related genes

The targets can and RNA hybrids analysis software were used to analyze the target relationship between miR-23b-3p and GH secretion related genes (GHRHR, SSTR2, LEF1, POU1F1, SSTR5 and CREB1). The results showed that the target gene of miR-23b-3p was POU1F1.

Verification of the target relationship between miR-23b-3p and POU1F1

In order to further verify whether there is a target relationship between miR-23b-3p and POU1F1, pirGLO-POU1F1-3'UTR normal or mutant vectors were co-transfected into CHO cells with miR-23b-3p mimics (miR-23b-3p-mi group) and mimics control substance (NC group), respectively. The change of luciferase activity was observed 48 h later, and the target relationship between miR-23b-3p and POU1F1 was analyzed by the changes of luciferase activity. The results showed that the luciferase activity in pirGLO-POU1F1-3'UTR normal plasmid was significantly decreased by adding miR-23b-3p mimics (Fig. 4a) ($P < 0.01$), but no inhibitory effect on that in the pirGLO-POU1F1-3'UTR mutant vector ($P > 0.05$) (Fig. 4b). It could be concluded that miR-23b-3p could bind and act on the 3'UTR region of POU1F1, and there is a target relationship between miR-23b-3p and POU1F1.

Effect of miR-23b-3p on transcription level of POU1F1 mRNA in pituitary cells of Yanbian yellow cattle

In order to verify the regulatory function of miR-23b-3p on POU1F1 gene, the primary cultured pituitary cells of Yanbian yellow cattle were transfected with mimics (miR-23b-3p group), mimics reference substance (NC group), inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p, and then the transcription level of POU1F1 mRNA was detected by qPCR. The results showed that the transcription level of POU1F1 mRNA in the miR-23b-3p-mi group was significantly lower than that in the NC group (Fig. 5a) ($P < 0.01$), while the transcription level of POU1F1 mRNA in the miR-23b-3p-in group was significantly higher than that in the iNC group (Fig. 5b) ($P < 0.01$). These results suggested that miR-23b-3p could regulate the transcription of POU1F1.

Effect of miR-23b-3p on POU1F1 protein expression level in pituitary cells of Yanbian yellow cattle

In order to further verify the effect of miR-23b-3p on POU1F1 protein expression in pituitary of Yanbian cattle, POU1F1 protein expression level was detected by Western-blot based on the transcription results of POU1F1 mRNA. The results showed that the POU1F1 protein expression level in the miR-23b-3p-mi group was significantly lower than that in the NC group ($P < 0.01$) (Fig. 6a), while the POU1F1 protein expression level in the miR-23b-3p-in group was significantly higher than that in the iNC group (Fig. 6b) ($P < 0.01$). These results suggested that miR-23b-3p could regulate the POU1F1 protein expression.

Discussion

As a posttranscriptional regulatory factor, there are few studies on the regulation of miR-23b-3p on animal growth, especially on the relationship between miR-23b-3p and GH secretion. Therefore, in order to determine whether miR-23b-3p is related to GH secretion, we first observed the effect of miR-23b-3p on GH mRNA transcription and protein expression at the level of pituitary cells in vitro. The results showed that miR-23b-3p could inhibit GH mRNA transcription and protein expression, which indicated that there was a close relationship between miR-23b-3p and GH. However, previous researchers have demonstrated that the main function of miRNA is to negatively regulate the expression of target genes[25, 26].

Therefore, we concluded that GH was not directly regulated by miR-23b-3p, and the target gene of miR-23b-3p needed further verification.

Some research showed that GHRHR, SSTR2, LEF1, POU1F1, SSTR5 and CREB1 were the main genes related to GH synthesis and secretion[22, 27–29]. Therefore, through bioinformatics analysis, the target relationship between miR-23b-3p and the 3'UTR region of the above genes was analyzed in this study. According to the prediction and judgment conditions of the target gene, we concluded that miR-23b-3p has a good matching relationship with the 3'UTR region of POU1F1. Some studies have shown that POU1F1 protein is a transcription factor specifically expressed in animal pituitary gland, which can promote GH transcription and expression and play an important regulatory role in animal growth and development[30, 31]. By constructing a dual luciferase reporter gene system, we found that although miR-23b-3p could significantly inhibit the luciferase activity of the normal plasmid, it had no effect on the mutant vector. The principle of luciferase reporter gene system is that the expression of luciferase gene in the system is down regulated or inhibited by miRNA directly acting on the 3'UTR region of the gene, which is the most direct evidence of the relationship between miRNA and targets. So it could be concluded that the 3'UTR of POU1F1 might be the target of miR-23b-3p.

After determining the relationship between miR-23b-3p and 3'UTR of POU1F1, we verified the effect of miR-23b-3p chemical synthesis mimics (miR-23b-3p-mi) and its corresponding inhibitors (miR-23b-3p-in) on POU1F1 mRNA and protein pituitary cells in vitro. The results showed that miR-23b-3p inhibited the expression of POU1F1 mRNA and protein at the level of pituitary cells. The results further demonstrated that there was a target relationship between miR-23b-3p and POU1F1, and miR-23b-3p had a negative regulatory effect on POU1F1. The results further proved that the main function of miRNA was to negatively regulate the expression of target genes, which was consistent with the previous findings[32]. In addition, this result also verified the prediction results of bioinformatics. However, the specific regulatory network and mechanism of miR-23b-3p regulating GH need further study.

Conclusion

The results demonstrated that miR-23b-3p could regulate GH secretion by targeting POU1F1. The result of this study will not only provide a theoretical basis for further study the mechanism of exosomal miRNA on regulating animal growth and development, but also provide a new target for the growth and development regulation of Yanbian yellow cattle.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Abbreviations

GH:Growth hormone; POU1F1: Pituitary specific transcription factor 1; GHBP: Growth hormone binding protein; GHR: Growth hormone receptor; IGFs: Insulin-like growth factors; IGFBP: Insulin-like growth factor

binding protein; GHRHR: Growth hormone releasing hormone receptor); SSTR2: Somatostatin receptor 2; LEF1: Lymphoid enhancer 1; SSTR5: Somatostatin receptor 5; CREB1: Cyclic adenosine phosphate effector binding protein 1).

Declarations

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Authors' contributions

1. and D.C. designed the experiments. J.J., A.L., and T. J. wrote the manuscript. R.Z., S.X.,and L.Y. performed animal and in vitro experiments and most analysis with the help of co-authors. The authors read and approved the final manuscript.

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Ethics declarations

Ethics approval

All procedures with animals received prior approval from the Animal Care and Use Committee of Yanbian University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Fig.1

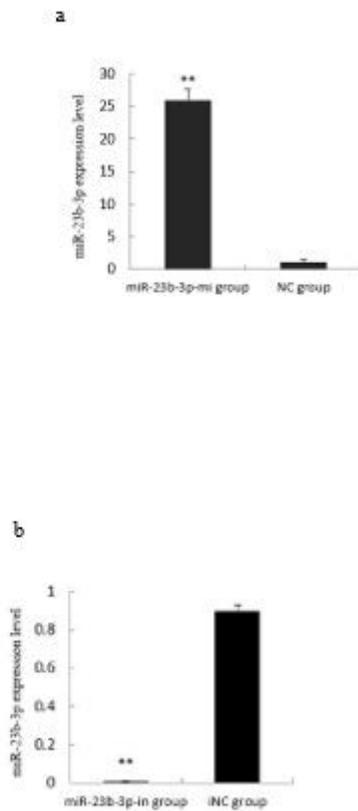
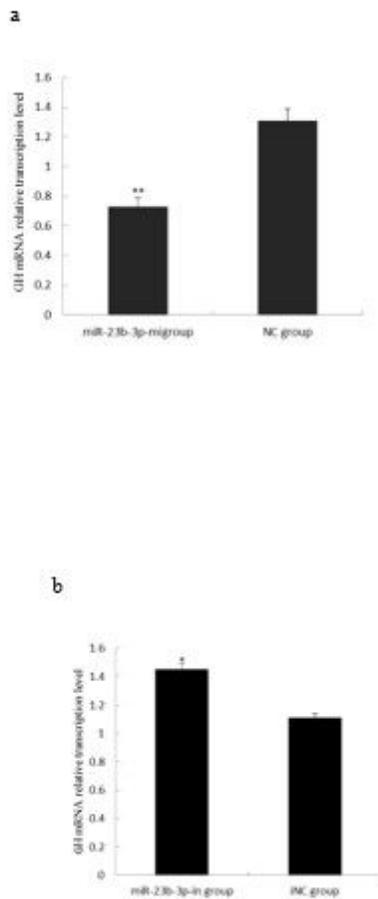


Figure 1

Expression level of miR-23b-3p in pituitary cells of Yanbian yellow cattle. (a) The expression level of miR-23b-3p in pituitary cells transfected with the mimics (miR-23b-3p group) and mimics reference substance (NC group) of miR-23b-3p. Compared with NC group, the column marked by ** showed significant difference ($P < 0.01$); (b) The expression level of miR-23b-3p in pituitary cells transfected with the inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p. U6 was used as an internal reference.

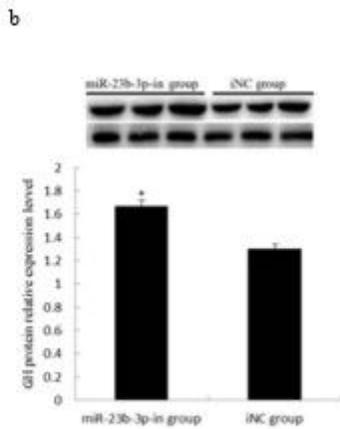
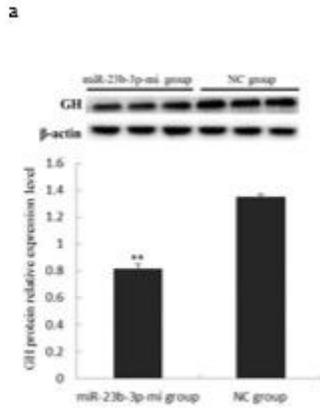
Fig.2



2

Figure 2

Effect of miR-23b-3p on GH mRNA transcription level in pituitary cells of Yanbian yellow cattle. (a) The relative transcription level of GH mRNA in pituitary cells transfected with miR-23b-3p mimics. Mimics (miR-23b-3p-mi group) and mimics reference substance (NC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking** showed extremely significant difference ($P < 0.01$); (b) The relative transcription level of GH mRNA in pituitary cells transfected with miR-23b-3p inhibitor. Inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking* showed significant difference ($P < 0.05$). β -actin was used as an internal reference.

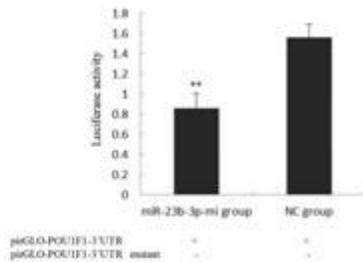


2

Figure 3

Effect of miR-23b-3p on GH protein expression level in pituitary cells of Yanbian yellow cattle. (a) The relative expression level of GH protein in pituitary cells transfected with miR-23b-3p mimics. Mimics (miR-23b-3p-mi group) and mimics reference substance (NC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking** showed extremely significant difference ($P < 0.01$); (b) The relative expression level of GH protein in pituitary cells transfected with miR-23b-3p inhibitor. Inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking* showed significant difference ($P < 0.05$). β -actin was used as an internal reference. Electrophoresis of the GH and β -actin gene fragment was from two separate gels.

a



b

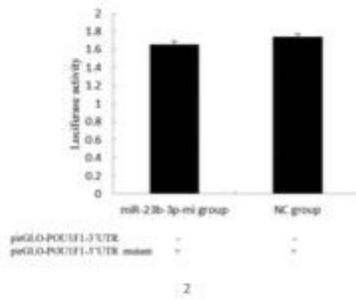
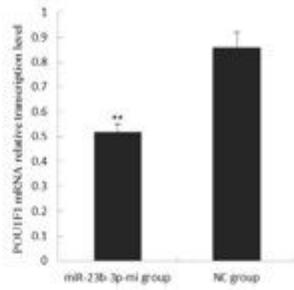


Figure 4

Luciferase activity determination. (a) The changes of luciferase activity after PirGLO-POU1F1-3'UTR normal plasmid co-transfecting with miR-23b-3p mimics (miR-23b-3p-mi group) and mimics reference substance (NC group) for 48 h. Compared with NC group, the column marking** showed extremely significant difference ($P < 0.01$); (b) The changes of luciferase activity after PirGLO-POU1F1-3'UTR mutant plasmid co-transfecting with miR-23b-3p mimics (miR-23b-3p-mi group) and mimics reference substance (NC group) for 48 h. Compared with NC group, the column without marking** or * showed no significant difference ($P > 0.05$).



b

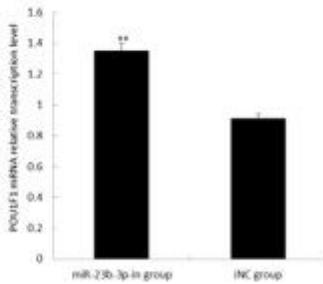
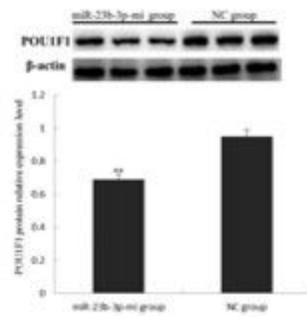
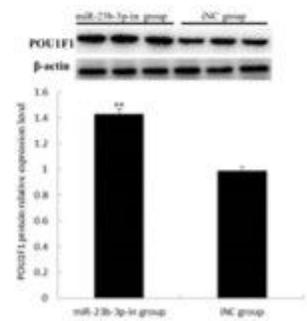


Figure 5

Effect of miR-23b-3p on transcription level of POU1F1 mRNA in pituitary cells of Yanbian yellow cattle. (a) The relative transcription level of POU1F1 mRNA in pituitary cells transfected with miR-23b-3p mimics. Mimics (miR-23b-3p-mi group) and mimics reference substance (NC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking** showed extremely significant difference ($P < 0.01$); (b) The relative transcription level of POU1F1 mRNA in pituitary cells transfected with miR-23b-3p inhibitor. Inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking** showed extremely significant difference ($P < 0.01$). β -actin was used as an internal reference.



b



2

Figure 6

Effect of miR-23b-3p on POU1F1 protein expression level in pituitary cells of Yanbian yellow cattle. (a) The relative expression level of POU1F1 protein in pituitary cells transfected with miR-23b-3p mimics. Mimics (miR-23b-3p-mi group) and mimics reference substance (NC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with NC group, the column marking** showed extremely significant difference ($P < 0.01$); (b) The relative expression level of POU1F1 protein in pituitary cells transfected with miR-23b-3p inhibitor. Inhibitor (miR-23b-3p-in group) and inhibitor reference substance (iNC group) of miR-23b-3p were transfected into pituitary cells of Yanbian yellow cattle, with three replicates in each group. Compared with iNC group, the column marking** showed significant difference ($P < 0.01$). β -actin was used as an internal reference. Electrophoresis of the POU1F1 and β -actin gene fragment was from two separate gels.