

Mir-183-96-182 cluster may not work together in bovine ovarian granulosa cell apoptosis

Keywords: mir-183-96-182 cluster, cattle, granulosa cell, follicle, apoptosis

Introduction

Follicle development in cattle undergoes two important processes, one is ovulation of the preovulatory dominant follicle, and the other is degeneration of anovulatory subordinate follicles Fortune et al.¹ The degeneration of anovulatory subordinate follicles also called follicular atresia, in which apoptosis of granulosa cell is the main mechanism.^{1,2} miRNAs which are ~22nt in length non-coding endogenous RNA molecules regulate gene expression in a sequence-dependent manner at posttranscriptional level Ambros.³ How these small molecules post-transcriptionally regulate apoptosis process and finally the follicles goes atresia is poorly understood. In our previous paper, miR-183 miRNA cluster which includes miR-183, miR-96 and miR-182 showed higher expression levels in dominant bovine follicles than in subordinates Ma et al.⁴ In this study, the miR-183-96-182 cluster will be transfected into bGCs separately to summarize the functions of this cluster in apoptosis.

Animal and GC collection

The ovaries were collected in local abattoir. The classify of the ovaries is based on the rules described by Ireland et al.⁵ Follicles ≤ 11 and ≥ 12 mm in diameter were classified as subordinate and dominant, respectively Spicer et al.⁶ The GCs were collected in the follicles before ovulation. The oocytes were removed using a mouth-operated micropipette under a microscope, and the GCs were resuspended in DMEM medium with 10% (V/V) Fetal Bovine Serum.

Apoptosis analysis of GCs

The transfection process is followed the Lipofectamine 2000 protocol. The 100 μ M miR-183, miR-96 and miR-182 mimics or control was transfected into 12-well culture plate. After 48h, the cells were enzymatically digested and washed with PBS. Then, the cells were measured through AnnexinV-FITC/PI double staining using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences Pharmingen) in accordance with the manufacturer's instructions. Results were analyzed using cell flow cytometry.

Statistical analysis

All experiments for miR-183-96-182 cluster or the control were conducted in triplicates. All data are presented as means \pm SE, and statistical evaluation of the data was conducted with SPSS 14.0. Statistical analysis was performed with the independent-sample Student's t-test to compare the two groups. Differences at $P < 0.05$ were considered statistically significant. To test the potential role of miR-183 members in GC apoptosis of the bovine follicles, the isolated bGCs were incubated in vitro and transiently transfected with miRNA mimics or NC. The apoptotic rate was estimated by Annexin V-FITC/PI double staining. Results indicated that miR-183-96-182 did not have the same trend in regulation of GC apoptosis, miR-183

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had no function to induce the apoptosis of bGCs ($P > 0.05$), miR-182 and miR-96 have the opposite result, miR-183 could increase the apoptosis rates of bGCs ($P < 0.01$), while miR-96 could decrease the apoptosis rates of bGCs ($P < 0.05$).

miR-183-96-182 cluster had the function in regulation of cell apoptosis Tang et al.⁷ and proliferation Zhang et al.⁸ With the GC aspirated from small growing follicles (3-5mm in diameter), Gebremedhn showed overexpression of miR-183-96-182 cluster promotes GC proliferation, they did not check the apoptosis rate of GC Gebremedhn et al.⁹ In our study, we did not find this cluster work together in apoptosis regulation, the reason needs further study. In conclusion, miR-183-96-182 cluster has diversiform regulation function in GC apoptosis, which may help to provide new clues in the research of follicular atresia.

Conclusion

In this study, cell flow cytometry was employed to test the function of miR-183-96-182 cluster in bovine ovarian granulosa cell apoptosis. miR-183-96-182 did not have the same trend in regulation of GC apoptosis, miR-183 had no function to induce the apoptosis of bGCs, miR-182 and miR-96 have the opposite result, miR-183 could increase the apoptosis rates of bGCs, while miR-96 could decrease the apoptosis rates of bGCs.

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Conflict of interest

Author declares that there is no conflict of interest.

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