miR-2382-5p regulates lipid metabolism via targetting NDRG2 in mammary epithelial cells of dairy cattle

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Introduction

microRNA (miRNA) is a class of single-stranded RNA molecules of about 22-24 nucleotides in length which regulate a variety of biological processes including lipid metabolism and triglyceride synthesis. Our study aimed to validate the regulatory relationship between miR-2382-5p and *NDRG2* at cellular level, and explore the regulatory mechanisms of miR-2382-5p and *NDRG2* in lipid metabolism in BMECs. This study provided basic data for the further understanding lipid metabolism in dairy cattle.

Result 1 miR-2382-5p inhibits lipid metabolism in BMECs

The data of quantitative RT-PCR showed that the expression of miR-2382-5p in BMECs significantly increased after transfection with miR-2382-5p mimics and significantly decreased after transfection with miR-2382-5p inhibitor, respectively. Furthermore, the triglyceride content of miR-2382-5p mimics transfected group was significantly reduced, whereas the triglyceride production was significantly elevated in miR-2382-5p inhibitor transfected group (Figure 1). Quantitative RT-PCR results showed that the expression levels of LPL and PPARGC1B were significantly up-regulated in BMECs transfected with miR-2382-5p mimics. The expression of HSL and PPARγ increased in both miR-2382-5p mimics and miR-2382-5p inhibitor groups, however, the expression levels of HSL and PPARγ were higher in miR-2382-5p mimics transfected cells. The expression of FASN showed no difference between Nc and miR-2382-5p mimics or miR-2382-5p inhibitor groups. The expression of CEBP-β and ACACB were up-regulated in both miR-2382-5p mimics and miR-2382-5p inhibitor groups, whereas the expression levels of CEBP-β and ACACB were decreased in BMECs transfected by miR-2382-5p mimics compare with cells transfected with miR-2382-5p inhibitor (Figure 2).

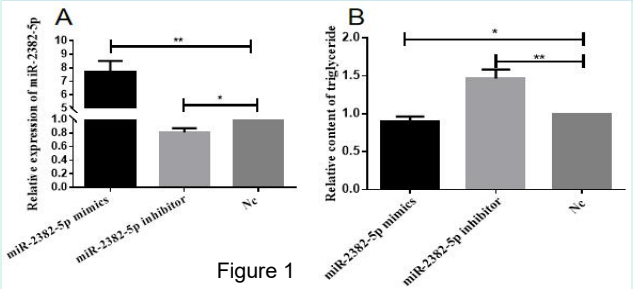

Figure 1

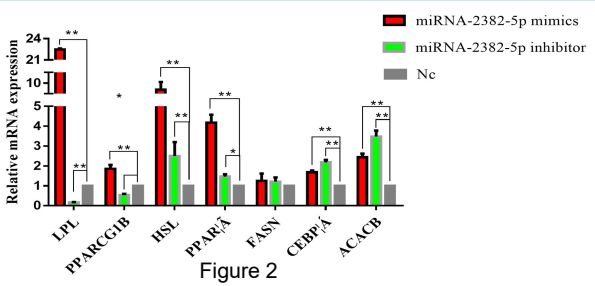

Figure 2

Result 2 miR-2382-5p negatively regulates the expression of NDRG2

Luciferase activity of BMECs transfected with *pmiR-BR-REPORT-NDRG2-WT* decreased compared with BMECs transfected with *pmiR-BR-REPORT-NDRG2-mut* and *pmiR-BR-REPORT*, respectively (Figure 3A). Data from quantitative RT-PCR showed that the expression of *NDRG2* mRNA significantly decreased in BMECs transfected with *miR-2382-5p mimics* and significantly increased with *miR-2382-5p inhibitor* when compared with BMECs transfected with *Nc* (Figure 3B).

Result 3 NDRG2 promotes triglyceride and cholesterol synthesis in BMECs

Quantitative RT-PCR and western blot results showed that transfection with *pBI-CMV3-NDRG2* up-regulated the expression of *NDRG2* mRNA and protein, whereas transfection with *pGPU6/GFP/Neo-NDRG2* down-regulated the expression of *NDRG2* mRNA and protein (Figure 4). In addition, the detection of triglyceride and cholesterol content in BMECs showed the overexpression of *NDRG2* significantly increased the synthesis of triglyceride and cholesterol, and the interference of *NDRG2* significantly inhibited the synthesis of triglyceride and cholesterol (Figure 5).

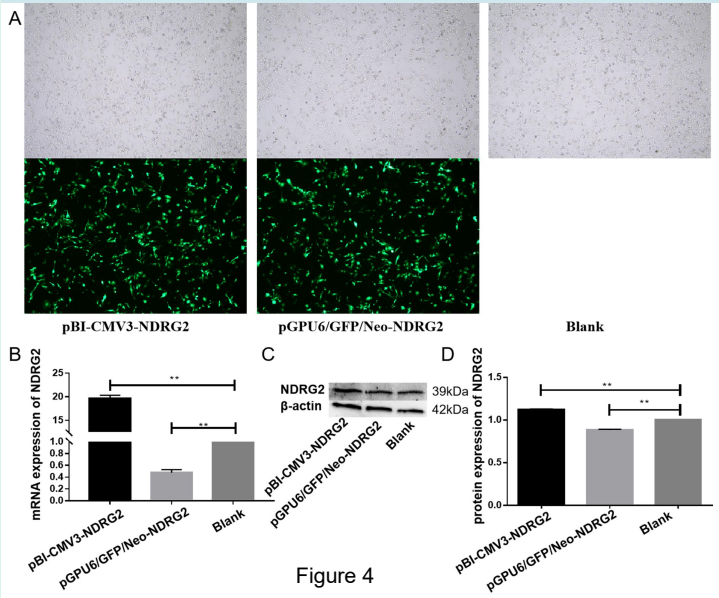

Figure 4

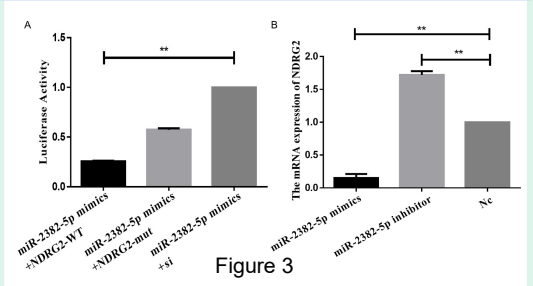

Figure 3

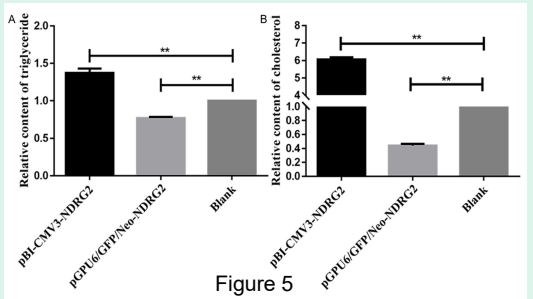

Figure 5

Conclusion

miR-2382-5p could negatively regulate lipid metabolism via regulating the genes associated with triglyceride decomposition, such as LPL, PPARGC1B, HSL, and PPARγ. miR-2382-5p could specifically inhibit the expression of target gene NDRG2 which in turn could increase triglyceride and cholesterol synthesis. The miR-2382-5p-NDRG2 signal pathway as a novel pathway involves in lipid metabolism in BMECs. Further, miR-2382-5p and NDRG2 could be potential targets for marker-assisted selection in molecular breeding to manipulate milk fat percentage.

Keywords: dairy cattle, bovine mammary epithelial cells, lipid metabolism, miR-2382-5p, NDRG2

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