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Full Length Article

Identification of Transport Stress miRNAs in Beef Cattle by High-Throughput Sequencing and Bioinformatics Functional Analysis

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Abstract

MicroRNAs (miRNAs) are endogenous small noncoding RNA molecules that regulate gene expression post-transcriptionally. They are involved in the regulation of growth, tumor development and stress survival. This study investigated the effect of 6 h of road transport stress on the miRNA status in liver of beef cattle. High-throughput sequencing results showed that 1515 known miRNAs and 2014 novel miRNAs were altered by transport stress; 2029 were upregulated and 1590 were downregulated miRNAs. Bioinformatics analysis of differentially expressed miRNAs predicted 21,946 target genes and 56 enriched gene ontology terms, with 44 enriched KEGG pathways, including immunization-related the MAPK signaling, focal adhesion, axon guidance, and cAMP signaling pathway. Twelve potentially stress-related miRNAs were selected and analyzed by high-throughput sequencing of the beef bovine liver at 0 and 6 h after transport. Differential expression of miRNAs was performed by bioinformatics analysis and verified by stem-loop fluorescence quantification. This study is the first to explore changes in liver miRNAs in beef cattle exposed to transport stress, providing insight into the molecular basis of beef cattle transport stress and the evolution of stress-related miRNAs. © 2020 Friends Science Publishers

Keywords: Beef cattle; Transport stress; miRNA; High-throughput sequencing

Introduction

As a result of the continuous improvement of intensive farming at home and abroad and the promotion of farms, the long-distance transportation of animals has become increasingly common. Transport is a common stressor that can reduce the immunity of beef cattle. This can affect growth and development, resulting in poor performance and low quality of livestock products, decreased disease resistance and significantly increased morbidity and mortality. Transported animals are exposed to different physical and psychological stimuli that can affect homeostasis and metabolism. Therefore, understanding the effect of stress on the health and performance of beef cattle is important.

Transportation-associated stress alters the levels of certain cytokines, heat shock proteins and acute phase proteins (Yu *et al.* 2009; Browning and Leite-Browning 2013; Wei *et al.* 2018). Changes in specific indicators during transport suggest that they could be used for the assessment of animal welfare during transport (Marques *et al.* 2016). These indicators are considered as reference markers for stress diagnosis. However, to date, a single indicator that can comprehensively and accurately assess the effect of stress has not been identified. In addition, the molecular mechanism underlying transport stress in beef cattle remains largely unclear. Biological systems activate different mechanisms to maintain their function in response to various stresses (Su *et al.* 2019). Emerging data suggest that stress conditions can alter the biogenesis of microRNAs (miRNAs), which can regulate gene expression at the post-transcriptional level and play crucial regulatory roles in a variety of biological processes, such as stress responses (Bartel 2009; Pritchard *et al.* 2012; Guo *et al.* 2018a). Advances in scientific research and equipment have improved our understanding of the relationship between transport stress and miRNAs. Deep sequencing has led to a dramatic increase in the discovery rate of novel miRNAs (Kozomara and Griffiths-Jones 2011); therefore, its use for detecting miRNAs associated with transport stress may enhance the exploration of miRNAs. Studies have identified miRNA changes in organisms in response to different stresses and used these to determine expression profiles using high-throughput sequencing technology. The expression of miRNAs in animals changes in response to environmental stresses, including heat stress and cold stress,

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among others (Yang *et al.* 2011; Yu *et al.* 2011; Rao *et al.* 2017). Three miRNAs (miR-22, miR-155 and miR-365) are upregulated during transport stress in turkeys, suggesting that the expression levels of these three circulating miRNAs may have diagnostic value in discriminating between stress from non-stress animals (Lecchi *et al.* 2016). Understanding miRNA expression patterns in transported animals may help elucidate the regulatory mechanism underlying transport stress responses, which would help design strategies to increase the resistance to transportation stress. In the present study, conserved and novel miRNAs were extracted from beef cattle liver that we identified under normal and transport conditions using deep sequencing (BGISEQ-500). Functional analysis showed that transportation stress-related miRNAs were involved in many biological processes. The study was the first to study the expression patterns of miRNA in the liver of transported beef cattle. This study constitutes a starting point to investigate the roles of miRNAs in beef cattle under transport conditions. This may lead to the identification of potential diagnostic biomarkers for transport stress, which would be beneficial for study on animal transport and aerospace.

Therefore, this study is based on the identification of transport stimulation, a common stress source in livestock and poultry breeding. Taking beef cattle as the research object, we used modern molecular biology techniques such as high-throughput sequencing technology and qRT-PCR to screen transport-stressed beef cattle liver miRNAs. We performed differential expression profiling, combined with bioinformatics technology, analysis of differential expression of miRNAs and their target genes related to transport stress, and used qRT-PCR technology to verify target miRNAs to obtain diagnostic markers for transport stress candidate miRNAs.

Materials and Methods

Animals and transport stress

Fifteen 1 year-old female beef cattle (320 ± 23 kg) were divided into three groups comprising 15 animals each: a control group (transport 0 h), a transport 3 h group, and a transport 6 h group. The animals were kept in a single paddock and provided with a feedlot ration. On the day of transportation, the temperature was required to be between −8°C and −18°C; the wind speed in the air was 4 mph; the weather was clear; and the cattle car travelled at a speed of 80 km/h. The transport groups of test beef cattle were transported from the farm to the slaughterhouse at 3 and 6 h. No food or water were provided during transportation. And all experimental protocols were performed in accordance with relevant guidelines and regulations of the Heilongjiang Bayi Agricultural University.

Sample collection

All animals were treated immediately following the transportation. The serum samples from all 15 animals were separated and used for assessment of the level of stress through markers using ELISA, including interleukin-1 (IL-1, EIAab Wuhan, China, E0563b), interleukin-6 (IL-6, EIAab Wuhan, China, E0079b), interleukin-8 (IL-8, EIAab Wuhan, China, E0080b), interleukin-10 (IL-10, EIAab Wuhan, China, E0056b), tumor necrosis factor-α (TNF-α, EIAab Wuhan, China, E0133b), cortisol (CORT, EIAab Wuhan, China, E0462Ge), heat shock protein 70 (HSP70, EIAab Wuhan, China, E0873b), haptoglobin (HP, EIAab Wuhan, China, E0817b), serum amyloid protein (SAA, EIAab Wuhan, China, E0885b), and C-reactive protein (CRP, Meimian, Jiangsu, China, MM-0021O1). The livers of all animals were also collected and stored at -80°C.

Deep sequencing

Three biological replicates were generated for each control and transported samples. Preparation of miRNA libraries was then performed according to the standard procedure at Shenzhen Genomics Institute (BGI BGISEQ-500, China).

Bioinformatics analysis

Target genes of differentially expressed miRNAs were predicted using RNAhybrid, miRanda, TargetScan, miRDB, miRwalk and mirTarBase intersection of four predicted results were considered reliable differentially expressed miRNA target genes for further analysis. Data were collected as previously described (Zhen *et al.* 2017). Specifically, the functions of target genes regulated by differentially expressed miRNAs in response to transport stress in biological processes and signaling pathways were first annotated against the gene ontology (GO) non-redundant (NR), UniProt and KEGG pathway databases. P-values were used for multiple test correction by false discovery rate (FDR) estimation.

Results

Transportation treatment

Temperature of the analyzed beef cattle: There were no significant differences in core temperature between the control group and the two transport groups (average 37.4°C, 37.5°C and 37.3°C, respectively).

Inflammatory cytokines and cortisol in transport stress

The concentrations of stress-related cytokines including IL-1, IL-6, IL-8, IL-10, TNF-α in the sera of the three groups were analyzed (Fig. 1). Compared with the 0 h group, the concentrations of IL-1 and IL-6 increased significantly after 6 h of transportation ($P < 0.05$) (Fig. 1A–B). Transport stress did not have a significant effect on serum IL-8 levels (Fig. 1C). Compared with the 0 h group, the level of IL-10 decreased significantly at 3 h ($P < 0.05$), whereas it

increased significantly at 6 h ($P < 0.05$) (Fig. 1D). Compared with the 0 h group, the concentration of TNF-α was significantly elevated at 6 h after transport stress ($P < 0.05$) (Fig. 1E). CORT concentrations increased significantly in both groups of transport stress ($P < 0.05$) (Fig. 1F).

HSP70 and acute phase proteins in transport stress

The stress-related HSP70 protein and acute phase proteins HP, SAA and CRP were measured in the sera of the three groups. The results showed that HP, CRP, and HSP70 were upregulated in the 6 h transport group (Fig. 2A, B and D), whereas SAA was significantly upregulated in the transport stress group at 3 and 6 h. (Fig. 2C).

Analyses of miRNAs

To identify miRNAs involved in the response of beef cattle to transport stress, six separate libraries of small RNAs were constructed including three liver samples from each group. The six libraries were subjected to high-throughput sequencing (BGISEQ-500 technology) and 0h (66 239 200, 74 906 090, 56 237 345) and 6 h (89 299 076, 113 211 460, 56 870 975) raw tag counts were obtained. The genomes mapping rates of all samples were stress group (94.19%, 95.00%, 95.30%) and transport group (93.69%, 94.20%, 93.783%), respectively (Table 2).

Differentially expressed miRNAs induced by transport stress

To identify transport stress-related miRNAs, the known and novel miRNA expression profiles were examined at 0 and 6h. The expression levels of 1515 known miRNAs and 2014 novel miRNAs were altered by transport stress. There were 2029 upregulated miRNAs and 1590 downregulated miRNAs, of which 1359 novel miRNAs were upregulated and 745 novel miRNAs were downregulated.

Prediction of target genes of transport stress responsive miRNAs

Two software programs were used in RNAhybrid and miRanda to identify the target genes of miRNAs and to determine the intersection of target genes as the final prediction result. A total of 22915 target counts were predicted for the 3842 differentially expressed miRNAs by the two prediction programs. At the intersection of the two software programs, 22895 target genes were predicted. To identify possible targets, taking the intersection targets with appropriate filter conditions such as minimum free energy (MFE), a score for further analysis was assigned. Finally, 21946 target genes were predicted for differentially expressed miRNAs. The combined target results were shown in Fig. 3.

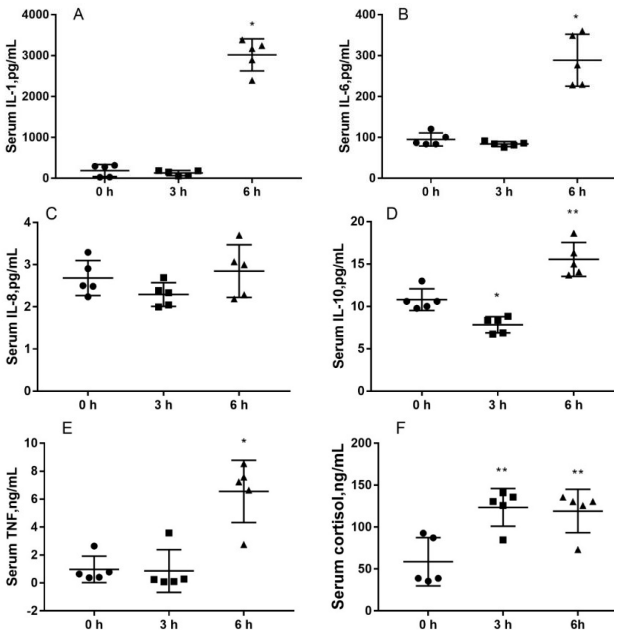

Fig. 1: Effect of transport stress on IL-1, IL-6, IL-8, IL-10 (A), TNF-α, CORT. Results are expressed as the mean ± SD (n = 5 per group) of three independent experiments. *$P < 0.05$, **$P < 0.01$

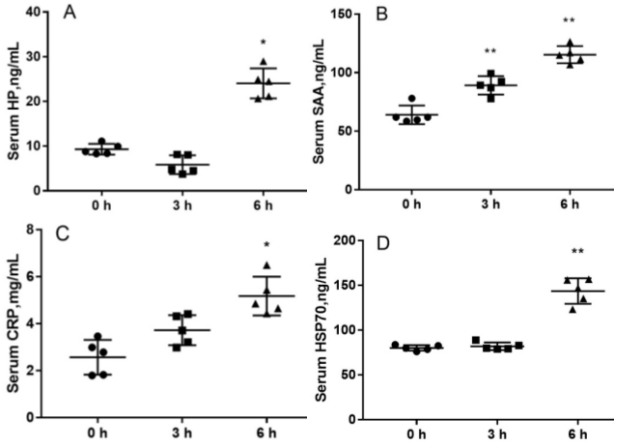

Fig. 2: Effect of transport stress on SAA, HP, CRP, and HSP70. Results are expressed as the mean ± SD (n = 5 per group) of three independent experiments. *$P < 0.05$, **$P < 0.01$

Analysis of target genes in transport stress related GO

We performed GO and KEGG pathway analysis on target genes of differentially expressed miRNAs to explore potential pathways regulated by these miRNAs. GO classification results showed that 21,946 target genes for differentially expressed miRNAs were aggregated into 56 GO terms. The top 20 terms classified into Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) were shown in Fig. 4A. There were 16 terms related to "cell components", of which the most abundant were related to

Table 1: List of primers used for amplification of miRNAs through qRT-PCR

miRNA	Primer	Primer sequence(5`-3`)
miR-194-5p	*RT*	*GAGGTATTCGCACTGGATACGACTCCACA*
	Forward	*ACGCGCGTGTAACAGCAACTCCA*
miR-455	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGATGTA*
	Forward	*CCGCGCGTATGTGCCTTTGGAC*
miR-199a	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACAGG*
	Forward	*CGCGCCCAGTGTTCAGACTA*
miR-31	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGCTAT*
	Forward	*ACGCGAGGCAAGATGCTGGC*
miR-186	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGCCCA*
	Forward	*CGCGCAAAGAATTCTCCTTT*
miR-152	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCCAAG*
	Forward	*CGCGTCAGTGCATGACAGAA*
miR-125b	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCACAA*
	Forward	*CGCGTCCCTGAGACCCTAAC*
miR-30a-5p	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGCTTC*
	Forward	*GCGTGTAAACATCCTCGACTG*
miR-122	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAAACA*
	Forward	*CGCGTGGAGTGTGACAATGG*
novel-mir-1624	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTTGGG*
	Forward	*CGCGCGTTCTCTAGTGTGTTTT*
novel-mir-2108	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAGAG*
	Forward	*CGCGTCATGCTGTGCTGTCT*
novel-mir-817	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCAGG*
	Forward	*CGTGGGTCGCGACCTCTC*
novel-mir-205	*RT*	*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCTCTG*
	Forward	*CGCAGGGAGGGTGGGC*
U6	*RT*	*CGCTTCACGAATTTGCGTGTCAT*
	Forward	*GCTTCGGCAGCACATATACTAAAAT*

Table 2: Summary of sequencing data for each sample and alignment statistics of tags aligned to the reference genome

Sample name	Raw tag count	Clean tag count	Mapped tag	Percentage (%)
0 h	66239200	61336192	57773194	94.19
0 h	74906090	71578918	67999638	95.0
0 h	56237345	52767940	50286171	95.3
6 h	89299076	84273044	78951593	93.69
6 h	113211460	106884997	100681164	94.2
6 h	56870975	53896496	50570840	93.83

*Percentage (%) = (total mapped tag number/ total clean tag number) *100%

cells, cell parts. Fifteen terms were associated with "molecular function", in which binding genes were the most abundant.

The KEGG public database is used to analyze the pathway enrichment of different genes. The results showed that the target genes of these differential miRNAs were involved in the MAPK signaling pathway, axon guidance, cAMP signaling pathway and focal adhesion; the top 20 enriched pathways were shown in Fig. 4B. In addition, we graphically displayed the KEGG enrichment analysis results, which classified genes into six top KEGG pathways (44 seconds). Mapping of the top 20 enriched pathways in descending order of the rich-factor revealed that the signal transduction pathway (ko04010) and focal adhesion pathway (ko04510) were important pathways under conditions of transport stress as shown in Fig. 5.

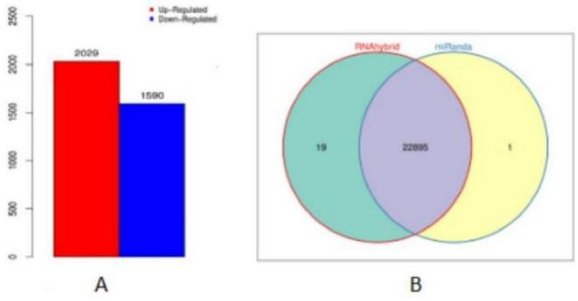

Fig. 3: Prediction of target genes of transport stress responsive miRNAs. (**A**). Statistical analysis of differently expressed miRNAs in transport beef cattle liver. (**B**). Venn statistics of different target gene prediction software of miRanda and RNAhybrid targets

Validation of miRNAs

We validated the results of high-throughput sequencing using a qRT-PCR method. Between the 0 h and 6 h groups,eight known miRNAs and four novel miRNAs were validated by stem-loop qRT-PCR between the 0 h and 6 h groups (Fig. 6). The primer sequences of eight known miRNAs and four novel miRNAs were showed on Table 1, that were used in qRT-PCR (Table 1). The results indicated that most of the miRNAs identified in this study were consistent with the high-throughput results and were considered to be believable.

Fig. 4: (A) GO enrichment analysis of all targets ($P < 0.01$, FDR < 5, and count > 10). **(B)** KEGG pathway annotation classification chart. The X axis shows the number of DEGs, the Y axis represents the second KEGG pathway terms. All second pathway terms are grouped into top pathway terms indicated in different colors

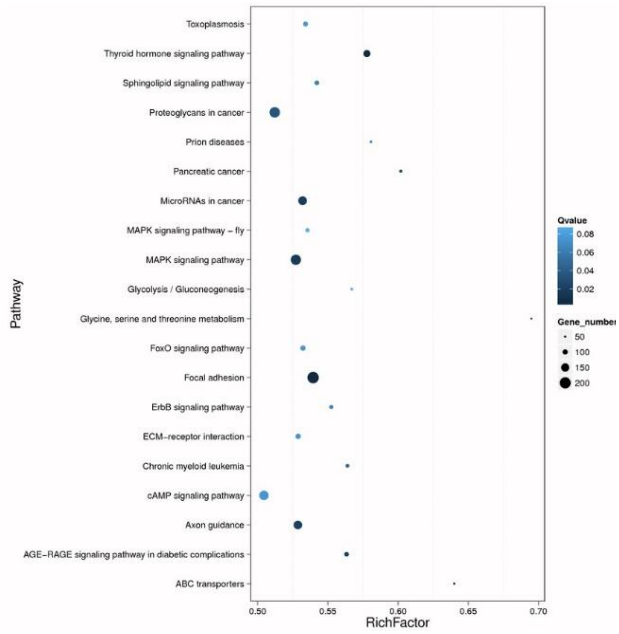

Fig. 5: Pathway enrichment statistics scatter plot. RichFactor is the ratio of different expression of miRNA target gene numbers annotated in this pathway term to all gene numbers annotated in this pathway term. A greater richFactor means a greater enrichment. The Q value is a corrected p-value ranging from 0 to 1, and a lower Q value means greater intensiveness. The top 20 enriched pathway terms are shown

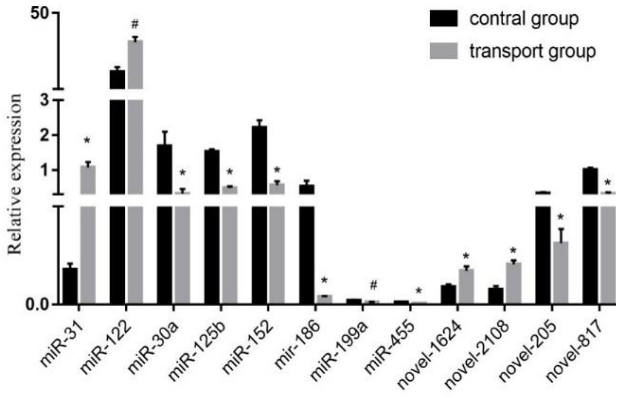

Fig. 6: The qRT-PCR results of 12 miRNAs in beef cattle liver. Asterisks (*) denote a relevant significant difference ($P < 0.01$) and pentagrams (#) denote a relevant significant difference ($P < 0.05$)

Bioinformatics analysis of miR-186 and miR-30a-5p

The target genes of bta-miR-186 were predicted using Targetscan, miRDB, miRWalk, and miRTarBase (Fig. 7A). The 37 candidate genes identified using the four software screens were used for further analysis (Fig. 7B). The results of the predicted binding sites of the target genes were shown in Fig. 7D. GO analysis showed that bta-miR-186 is involved in nine biological processes, namely biological regulation, cellular processes, developmental processes, metabolic processes, multicellular organism processes and responses to stimuli, among others. These biological processes may play an important role in regulation, metabolism, and response to stimuli (Fig. 7C).

The target genes of bta-miR-30a-5p were predicted by Targetscan, miRDB, miRWalk, and miRTarBase (Fig. 8A). The 48 candidate genes identified using the four software screens were used for further analysis (Fig. 8B). The results of the predicted binding sites of the target genes are shown in Fig. 8D. GO analysis showed that bta-miR-30a-5p is involved in 11 biological processes, including biological adhesion, biological regulation, developmental process, immune system process, locomotion, metabolic process, multicellular organismal process, and responses to stimuli, among others. These biological processes may also play an important role in the response to stimuli (Fig. 8C).

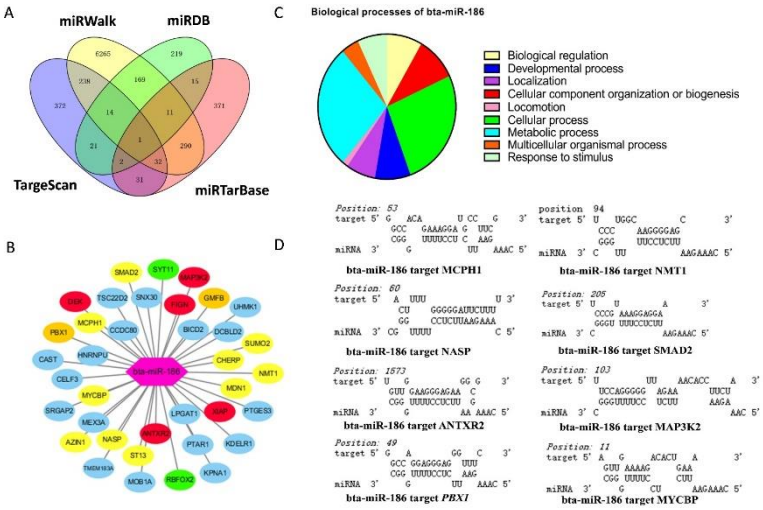

Fig. 7: Prediction of miR-186 target genes and biological functions. (**A**) Wayne map of target genes of miR-186. (**B**) Target genes of bta-miR-186. The red sections represent target genes for response to stimulus. The yellow sections represent target genes for metabolic process. The green sections represent target genes for multicellular organismal process. The orange sections represent target genes for developmental process. (**C**) Biological processes affected by bta-miR-186. (**D**) Identification of potential mRNAs (MCPH1, NMT1, NASP, SMAD2, ANTXR2, MAP3K2, PBX1, and MYCBP) that are direct targets of bta-miR-186. All the target sites were predicted using RNAhybrid

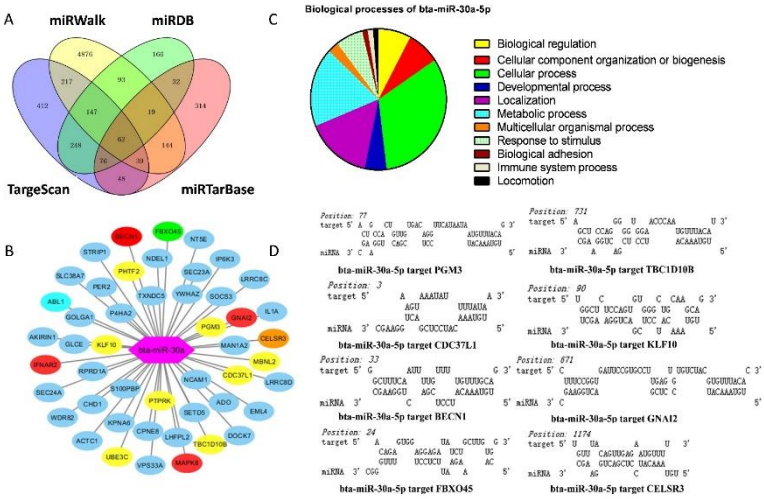

Fig. 8: Prediction of miR-30a-5p target genes and biological functions. (**A**) Wayne map of target genes of miR-30a-5p. (**B**) Target genes of bta-miR-30a-5p. The red sections represent target genes for response to stimulus. The yellow sections represent target genes for metabolic process. The green sections represent target genes for multicellular organismal process. The orange sections represent target genes for developmental process. The purple sections represent target genes for immune system process. (**C**) Biological processes affected by bta-miR-30a-5p. (**D**) Potential mRNAs (PGM3, TBC1D10B, CDC37L1, KLF10, BECN1, GNAI2, FBXO45, and CELSR3) that are direct targets of bta-miR-30a-5p. All the target sites were predicted using RNA hybrid

Discussion

Body temperature changes in beef cattle induced by transport stress are often not detected. The core temperature of animals increases in response to transport stress; however, in the present study, no temperature changes were detected. Considering that the body temperature regulation system of beef cattle was affected by the transport stress and a variety of factors are involved in the regulation, we believe that the transport stress response not reflects changes in body temperature.

In a preliminary set of experiments, we measured the levels of cytokines (IL-1, IL-6, IL-8 and IL-10) in beef cattle serum. We found that the cytokine levels were significantly higher in beef cattle transported for 6 h than in the 0h group. The change in cytokines during 3 h of transport was smaller

than that at 6 h of transport. CORT and TNF levels were detected in all groups analyzed, although the levels varied considerably between the groups, especially between 0 and 6 h.

Acute-phase proteins and heat-shock proteins are often used as indicators to assess stress. In the present study, there were significant changes in acute phase proteins and HSP70 in beef cattle serum at 6 h after transport. SAA was the most abundant protein. The analysis of blood biochemical indicators contributes to our understanding of the mechanism underlying transport stress and provides possible solutions. The current research on blood physiological indicators focuses on blood hormones, cytokines, and blood proteins. In preliminary experiments, stress led to changes in serum cytokines, acute phase proteins, and HSP70 and transportation for 6 h for beef cattle caused a stress response.

Transport is a common stressor that can cause serious adverse reactions in the body. The present study is the first to explore miRNA changes in transport-stress beef cattle livers and will provide insight into the molecular basis of beef cattle transport stress and the evolution of transport stress related miRNAs. miRNAs are important players in the strong adaptive response to animal stress because of their ability to fine-tune gene expression. The role of miRNAs in stress responses caused by environmental changes has been studied in mice (Guo *et al.* 2018b), bovines (Palma-Vera *et al.* 2015), chicken, and other mammals. However, the study of liver miRNAs in beef cattle under transport stress is not comprehensive. The results showed that transport stress altered the expression of 1515 known miRNAs in the liver of beef cattle and new miRNAs in 2014. There were 2029 upregulated miRNAs and 1590 downregulated miRNAs, of which 1745 new miRNAs were downregulated and 359 new miRNAs were upregulated. In a previous study (Palma-Vera *et al.* 2015) it was shown that 22nt may be the typical size of miRNAs in bovine species. Our results were consistent with prior data.

Identification of miRNAs and their target genes is the basis for understanding the physiological functions of miRNAs. There are important relationships between miRNAs and various stresses. Various studies have shown that stress can lead to the activation of the immune system (Zhang *et al.* 2017). When transport stress occurs, the gene expression level of turkey immune-related miRNAs changes, affecting the immune status of the turkey (Lecchi *et al.* 2016). Many animal miRNAs have been reported in miRbase, and some of the physiological functions of miRNAs have been revealed in related studies of cows exposed to heat stress (Zheng *et al.* 2014) or oxidative stress (Jacometo *et al.* 2015). Research on the connection between the postpartum cow liver and miRNAs showed that miRNAs are regulatory factors involved in mammalian metabolic and stress responses (Fatima *et al.* 2014; Chen *et al.* 2017). Nonetheless, there are no studies analyzing miRNAs related to transport stress in beef cattle liver.

To determine the target genes affected by miRNAs, GO enrichment analyses were performed, which revealed that the processes significantly altered by transport involved systems development, immune system progress, metabolic progress, and binding. KEGG pathway enrichment analysis showed that the target genes of differentially expressed miRNAs in transport stress beef cattle liver predominantly participated in MAPK signaling, thyroid hormone signaling, focal adhesion, the cAMP signaling pathway and pathways involved in cancer proteoglycans.

The multiple MAPK pathways present in all eukaryotic cells enable coordinated and integrated responses to diverse stimuli including environmental stresses (Kyriakis and Avruch 2012). Activation of the MAPK pathway has an important effect on cell physiology , which plays a significant role in coordinating gene transcription, protein biosynthesis, cell cycle control, apoptosis and differentiation (Rincon and Davis 2009; Keshet and Seger 2010; Rose *et al.* 2010). The MAPK signaling pathway plays an important role in various stresses, and its network regulates cell cycle progression and cell survival or death responses following a variety of stresses (Darling and Cook, 2014; Sui *et al.* 2014). P38 is one of the four MAPK subfamilies and is primarily involved in stress and inflammatory responses (Wu *et al.* 2006). The MAPK pathway can be activated by extracellular stimuli, such as oxidative stress. The pathways are also activated in response to stimulation by growth factors, inflammation, and cytokines. Once activated, MAPK plays an important role in converting extracellular stimuli into a wide range of cellular responses, including proliferation, differentiation, and senescence (Olsen *et al.* 2012; Yang *et al.* 2013). JNK, one of the MAPK families, is activated in response to various stress signals, including heat stress (Wijayagunawardane *et al.* 2015) and transport stress (Kyriakis *et al.* 1994; Dhanasiri *et al.* 2013; Wan *et al.* 2016).

Previous studies show that miR-122 is the most abundantly expressed miRNA in the liver of beef cattle and miR-30a-5p and miR-199a-3p are involved in heat stress when cows are subjected to heat stress (Zheng *et al.* 2014). Certain miRNAs are tissue specific. When bovine miRNAs are also conserved in other species, such miRNAs can serve as potential molecular markers of evolution.miR-125b is abundant in 11 tissues, and miR-122 is predominantly expressed in the liver (Jin *et al.* 2009).

Conclusion

In conclusion, this study found that a speed of 80 km/h and road transport for 6 h are suitable conditions to construct a beef cattle transport stress model. We screened 3,619 miRNAs differentially expressed in response to transport stress, and their target genes were involved in important pathways such as the MAPK signaling pathway. miR-186 and miR-30a-5p were identified as potential diagnostic markers for stress candidates. We examined the effects of transport stress on the serum stress index of beef cattle and miRNAs of beef cattle liver, which provided basic data for

further study on the molecular mechanism and control measures of beef cattle transport stress.

Acknowledgments

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