INVESTIGATION OF SIGNIFICANT MICRORNA-MRNA PAIRS ASSOCIATED WITH NONSPECIFIC DIGESTIVE DISORDER IN RABBITS

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Abstract: Nonspecific digestive disorders (NSDD) are one of the major intestinal problems in rabbit, with considerable economic losses in industrial rabbit farms. MicroRNAs (miRNAs), as small non-coding RNAs, have significant biological involvement in intestinal disorders. In this study, we investigated the expression levels of 25 genes and 25 miRNAs in ileum, rabbit *sacculus rotundus* (RSR) and colon tissues from 9 rabbits with different severity of NSDD. These molecules have been found to be related to NSDD or inflammatory bowel disease, which will help recognise the miRNA-mRNA pairs. Finally, 108 possible pairs of miRNA-mRNA pairs with an anti-correlation were identified by Pearson's correlation analysis between differentially expressed 25 miRNAs and 23 mRNAs. Ninety-five of these miRNA-mRNA pairs were hitherto unexplored, and their roles in NSDD biology require further elucidation. Our results give a clue to the potential miRNA-mRNA pairs for the NSDD that can further improve the understanding of the pathogenesis of NSDD in rabbit.

Key Words: nonspecific digestive disorders, rabbit, miRNAs, gene regulation, anti-correlation.

INTRODUCTION

Nonspecific digestive disorders (NSDD) are one of the major intestinal diseases in rabbit, which result from no clear intestinal pathogenic agents. NSDD causes considerable economic losses in industrial rabbit farms, with a mortality rate of about 10% (DeCubellis and Graham, 2013). Typical NSDD are characterised by stomach swelling, effusion and watery content, dilatation, impaction, congestion and mucus in the intestinal tract, particularly the infiltration of inflammation cells into the intestinal tract (Bennegadi *et al.,* 2001). It is well known that NSDD could be induced by several environmental factors, such as coccidiosis, bacterial infection, fibre-deficient diet, sanitary status and epizootic rabbit enterocolitis (Marai *et al.,* 2010). Meanwhile, individual genetic variation in susceptibility to NSDD has also been observed in recent years (Bartel, 2004; Garreau *et al.,* 2008; Zhang *et al.,* 2011; Chen *et al.,* 2013a; Liu *et al.,* 2013; Yang *et al.,* 2013b; Zhang *et al.,* 2013).

Previous studies have attempted to identify genetic factors influencing the rabbit immune system so as to provide insights into NSDD development. Toll-like receptors (*TLRs*) and nucleotide-binding oligomerisation domain-like receptors (NOD-like receptors, *NLRs*) are the major pattern recognition receptors (PRRs), which play essential roles in the activation of innate and adaptive immune responses. *TLR4* is a TLR which is responsible for pathogen recognition and activation of host innate immunity. It was found to be associated with genetic resistance against digestive disorders in New Zealand White rabbits (Zhang *et al.,* 2011). As a pivotal cytosolic adaptor downstream of *TLR4*, the genetic variant of myeloid differentiation factor 88 (*MyD88*) is significantly associated with NSDD and its mRNA expression appears to be positive correlated with the susceptibility to NSDD in rabbit (Chen *et al.,* 2013a). *NOD2* is a NLR which plays a role in the immune response by recognising bacterial molecules and inducing pro inflammatory cytokines and chemokines via nuclear transcription factor nf-kappa B (NF-κB)-based pathway. A synonymous mutation (c.2961C>T) in *NOD2* gene was significantly associated with susceptibility to NSDD in rabbit by genetic association and gene expression analysis (Zhang *et al.,* 2013). In contrast, *NLRP12* (NACHT, LRR and PYD domains-containing

protein 12), another member of the NLRs, inhibits the secretion of pro inflammatory cytokines and chemokines via NF-κB-based pathway. The *NLRP12* gene polymorphisms of c.1682A>G in rabbit had also been proposed as significantly associated with susceptibility to NSDD (Liu *et al.,* 2013).

The Janus tyrosine kinase (JAK)-signal transducer and activator of transcription (STAT) pathway are acknowledged as the critical signalling pathways downstream of cytokine receptors involved in immune regulatory functions. Recently, using multifactor dimensionality reduction method, researchers showed that *Tyrosine Kinase 2 (TYK2)*, *Janus kinase 1 (JAK1)*, and *signal transducer and activator of transcription 3 (STAT3)* genes and their gene-gene interactions play an important role in the genetic susceptibility to NSDD in rabbit (Fu *et al.,* 2014, 2015). In addition, some immunogenic biomarkers illustrated in inflammatory bowel diseases (IBD) may also be considered as related to NSDD-susceptibility genes.

MiRNAs are one family of small non-coding protein RNAs that regulate gene expression by causing mRNA degradation and inhibiting protein translation (Bartel, 2004). They are known to play important roles in regulating diverse biological process, including organ development, carcinogenesis and immune responses (Bartel, 2004). Recent studies have reported that miRNAs were strongly implicated in the pathogenesis of many diseases, such as bronchopulmonary dysplasia, heart failure and several types of cancer (D'Angelo *et al.,* 2016). Among them, the role of miRNAs in the development and regulation of the human IBD has been detected in several cases (Kalla *et al.,* 2015). For instance, the miR-106b and miR-93 was overexpressed in inflamed tissues of patients with Crohn's disease, which disrupted the autophagy pathway and bacterial clearance of AIEC (adherent-invasive *Escherichia coli*) by repressing *ATG16L1* (autophagy related 16 like 1) mRNA translation (Lu *et al.,* 2014). Similarly, miR-30c, miR-130a and miR-142-3p have also been shown to negatively regulate *ATG16L1* and autophagy (Nguyen *et al.,* 2014; Zhai *et al.,* 2014). To our knowledge, however, there are no studies on the relationship between miRNAs and mRNAs related to rabbit NSDD. In this study, we selected 25 genes and 25 miRNAs, which were found to be related to NSDD or IBD. So, we investigated the expression levels of these molecules in ileum, rabbit *sacculus rotundus* (RSR) and colon tissues among 3 different groups, to identify the miRNA-mRNA pairs that appear to be associated with rabbit NSDD.

MATERIALS AND METHODS

Ethics statement

All the procedures in this study were approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University, Sichuan, China (DKY-B20090908).

Inducing NSDD and preparation of tissues

A total of 60 healthy growing rabbit bucks with similar body weight (871±9 g) and at the age of 49 d were randomly selected from the experimental research farm at Sichuan Agricultural University. These 60 rabbits were housed one animal per cage in the same environment and provided with food and water under the same conditions, and were unrelated within 3 generations. With a 7-day dietary transition, these rabbits were fed fibre-deficient diet (crude fibre[CF]=8.89%, in contrast to the standard diet of CF=15.16%, Table 1), which can induce NSDD in growing rabbit (Gidenne *et al.,* 2000; Bennegadi *et al.,* 2001). From the age of 49 d to 70 d, they were carefully observed twice a day with precise records for all clinical signs of NSDD, such as reduced feed intake, diarrhoea, constipation (caecal impaction) and presence of mucus in excreta. At 70 d of age, all rabbits were slaughtered by intravenous administration of sodium pentobarbital and subsequently subjected to histopathological examinations and an autopsy.

Finally, 41 rabbits were successfully sampled and classified into 3 groups with different disease severity based on clinical signs and gastrointestinal tract symptoms of NSDD. There were 7 in the healthy group (without any symptoms), 10 in the mid NSDD group (laxativeness, intestinal flatulence and mucus) and 24 in the severe NSDD group (severe diarrhoea and dehydration, intestinal flatulence and bleeding, bowel wall thickening, caecal hyperaemia and caking). For these individuals, ileum, RSR and colon were removed and immediately frozen in liquid nitrogen and stored at –80°C for further use.

Table 1: Ingredients, chemical composition and nutritional value of experimental diets.

	Normal diet	Fibre-deficient diet
Diets ingredients (%)		
corn	15.8	25.8
wheat bran	17.0	19.8
wheat red dog	5.0	11.0
corn bran	5.0	10.8
rice bran	3.0	-
soybean meal	9.0	14.9
rapeseed meal	4.0	5.0
purple alfalfa meal	38.0	9.0
calcium powder	0.9	1.0
calcium hydrogen phosphate	1.1	1.5
salt	0.3	0.3
L-lysine	0.125	0.125
DL-lysine	0.075	0.075
mineral elements and vitamins additive	0.7	0.7
Nutritional value		
digestible energy (MJ/kg)	11.06	11.50
crude protein (%)	14.65	14.26
crude fibre (%)	15.16	8.89
acid detergent fibre (%)	21.19	13.38
neutral detergent fibre (%)	35.71	29.21

Nutritional value was estimated using nutritional value of feed ingredients.

MiRNA and gene expression analysis

In this study, 9 samples were used for mRNA and miRNA expression analysis, with 3 samples randomly selected for each group. Total RNA was isolated using RNAiso Plus reagent (TaKaRa, Japan). Prepared RNA quality (OD260/OD280:1.9~2.1) and integrity were confirmed by Nanodrop 2000 (Thermo Scientific, USA) and denaturing gel electrophoresis. cDNA was synthesised with SYBR® PrimeScriptTM miRNA RT-PCR Kit (TaKaRa, Japan) or PrimeScript® RT reagent Kit with gDNA Eraser (TaKaRa, Japan) according to the manufacturer's instructions, respectively. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using Premix Ex TaqTM II (TaKaRa, Japan) or SYBR® PrimeScriptTM miRNA RT-PCR Kit (TaKaRa, Japan) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-red) in a total reaction volume of 12.5 µL. The reaction condition of qPCR was: 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 1 min. The primers to amplify mRNAs and miRNAs were designed by Primer3 online-software and picked from miRBase database (http://www.mirbase.org/index.shtml), which were present in Table 2 and Table 3. All reactions were performed in triplicate for every sample. The *TBP* and *U6* genes were used as internal control to normalise the mRNA and miRNA data, respectively.

Statistical analysis

The comparative $2^{-\Delta t}$ method was used to analyse relative changes in gene expression levels for data normalisation. Differences among groups in one tissue were analysed using the one-way ANOVA with Duncan's multiple-range test, and significant differences ($P<0.05$) are indicated by different lowercase letters. Correlation between mRNA and miRNA were analysed using Pearson's correlation method, and significant correlation coefficients ($P<0.05$) are marked with an asterisk.

RESULTS

Differentially expressed mRNAs and miRNAs

IBD or NSDD-related 25 genes and 25 miRNAs were retrieved from published papers (Li *et al.,* 2011; Feng *et al.,* 2012; Ghorpade *et al.,* 2013; Koukos *et al.,* 2013; Liu *et al.,* 2013; Nata *et al.,* 2013; Yang *et al.,* 2013a, 2013b;

Table 2: Primers for mRNA of protein coding genes.

Gene symbol	Description	Primer sequences(5'-3')	Product size (bp)	MT (°C)
ATG16L1	Autophagy Related Protein 16 Like Protein 1	F:GACGCTCAGTCTCTTCCTTCC R:GGTTGGCACCCTCACTTCTT	83	61
ATG5	Autophagy Related Protein 5	F:GCCATCAATCGCAAACTCAT R:AGCCACAGGACGAAACAGC	123	61
DEFB135	βEDefensin 135	F:GTGGTCATCGTCTCGCTCTCC R:GCAAGTCCCTTTCAATCGCC	102	64
IFN-γ	Interferon-γ	F:TTGGGTTCTTACGGCTGT R:TGTTGTCACTCTCCTCTTTCC	151	57
IκBα	Nuclear Factor Kappa B Inhibitors Predominate	F:AGTCCAGGCAACAAAAGAGGT R:GGAAGCGGCGGTATGAATC	146	61.5
IL-10	Interleukin-10	F:TAAGGGTTACCTGGGATGC R:CCTGATGGCTGGACTGTG	97	58
IL-18	Interleukin-18	F:AAATCTCCATCATACGAAACC R:CGGGCATATCCTCAAACAC	81	57
IL-1β	Interleukin-1β	F:GCGTGATGAAAGACGATAAACCTAC R:TGGGGAACTGGGCAGACTC	144	62.5
IL-22	Interleukin-22	F:CTGTTCCCCGAGTCTGATAG R:CATTGGCTGAGATTGTTGC	86	57.5
IRGM	Immune related guanosine 3 phosphatase	F:GGTGTCCCAGATGCCAGTCA R:GCCTCCTCCTCGTGTCCTAT	102	62.5
JAK1	Protein-Tyrosine Kinases 1	F:ACCGAGGACGGAGGAAAC R:ACTGCCGAGAACCCAAAT	163	59
JAK2	Protein-Tyrosine Kinases 2	F:CTTTCAGAGCCATCATACGAG R:TTTTTCACAGCCACCACCT	259	58
NLRP12	Nucleotide-Binding Domain Leucine-Rich Repeat12	F:GCTGGCCGAATACGGGTT R:CAGGTAGCTCCTCGTCTCCT	94	61
NLRP3	Nucleotide-Binding Domain Leucine-Rich Repeat 3	F:TGAGGAAGAGGACACGGGACG R:GAGCCTGGTGGACCTGATTGC	92	66.5
NOD2	Nucleotide Binding Oligomerisation Domain 2	F:CCTTTGACGGCTTTGATGAGT R:GGCGTTCTTTAGGAGGTTGC	128	61
NP-5	Corticostatin-6	F:AAACAGGATGAAACCTCCCCTCTTG R:GTGTGTGGCGGACTCCATTGATTG	133	67.5
NUMB	NUMB, endocytic adaptor protein	F:GGAAGCGGCGGTATGAATC R:ATGGCAGTGGGTGGAGGAT	110	62.5
RHOB	Ras homolog family member B	F:CGGACTCGCTGGAGAACAT R:GGTAGTCGTAGGCTTGGATGC	204	61
SIAH2	Siah E3 ubiquitin protein ligase 2	F:CTAACGCCCAGCATCAGGA R:GGAGTAGGGACGGTATTCACAG	150	61
SMAD2	SMAD family member 2	F:ATTACATCCCAGAAACACCACC R:GGTTCTGAATAAGTAACTGGCTGC	169	60
SOCS-1	Suppressor of cytokine signalling 1	F:GCAGCCGACAATGCAGTCT R:GCGTGCGAGCGAAATGTA	134	62
STAT3	Signal Transducer and activator of transcription 3	F:GGGTGGAGAAGGACATCAGTG R:AAACTTGGTCTTCAGGTATGGG	263	60.5
TLR4	Toll Like Receptor 4	F:GAGCACCTGGACCTTTCAAATAAC R:GAACTTCTAAACCACTCAGCCCTTG	235	62.5
TNF	Tumour necrosis factor	F:CACTTCAGGGTGATCGGC R:TGCGGGTTTGCTACTACG	143	59
ZEB1	Zinc Finger E-box Binding Homeobox 1	F:ATACAAACACCACCTAAAAGAGCAC R:GATGGCGAGGAACACTGAGAC	195	61
TBP*	TATA-box binding protein	F:GACCCCTATTACTCCTGC R:TTTACAACCAAGATTCACTG	94	51.5

Table 3: Primers for miRNAs.

miRNA symbol	Primer	miRNA symbol	Primer
miR-10a	TACCCTGTAGATCCGAATTTGTG	miR-141	TAACACTGTCTGGTAAAGATGG
miR-21	TAGCTTATCAGACTGATGTTGA	miR-142-3p	TGTAGTGTTTCCTACTTTATGGA
miR-29a	TAGCACCATCTGAAATCGGTTA	miR-146a	TGAGAACTGAATTCCATGGGTT
miR-29b	TAGCACCATTTGAAATCAGTGTT	miR-146b	TGAGAACTGAATTCCATAGGCT
miR-30c	TGTAAACATCCTACACTCTCAGC	miR-150	TCTCCCAACCCTTGTACCAGTG
miR-31	AGGCAAGATGCTGGCATAGCT	miR-151-5p	TCGAGGAGCTCACAGTCTAGT
miR-106a	AAAAGTGCTTACAGTGCAGGTAG	miR-155	TTAATGCTAATCGTGATAGGGGT
miR-106b	UAAAGTGCTGACAGTGCAGAT	miR-192	CTGACCTATGAATTGACAGCC
miR-122	TGGAGTGTGACAATGGTGTTTG	miR-195	TAGCAGCACAGAAATATTGGC
miR-124	TAAGGCACGCGGTGAATGCC	miR-199a-5p	CCCAGTGTTCAGACTACCTGTTC
miR-126	TCGTACCGTGAGTAATAATGCG	miR-200b	TAATACTGCCTGGTAATGATGA
miR-130a	CAGTGCAATGTTAAAAGGGCAT	miR-362-3p	AACACACCTATTCAAGGATTCA
miR-132	TAACAGTCTACAGCCATGGTCG	U6[*]	CAAGGATGACACGCAAATTCG

Zhang *et al.,* 2013; Fu *et al.,* 2014; Lu *et al.,* 2014; Carethers *et al.,* 2015; Fu *et al.,* 2015; Kalla *et al.,* 2015), and their expression levels were then detected by RT-qPCR method in ileum, RSR and colon tissues. The relative expression level of the genes and miRNAs are shown in Figure 1 and Figure 2, in which the highest expression one is set to 1. In total, we detected 23 genes and 25 miRNAs significantly expressed among 3 groups in at least one tissue.
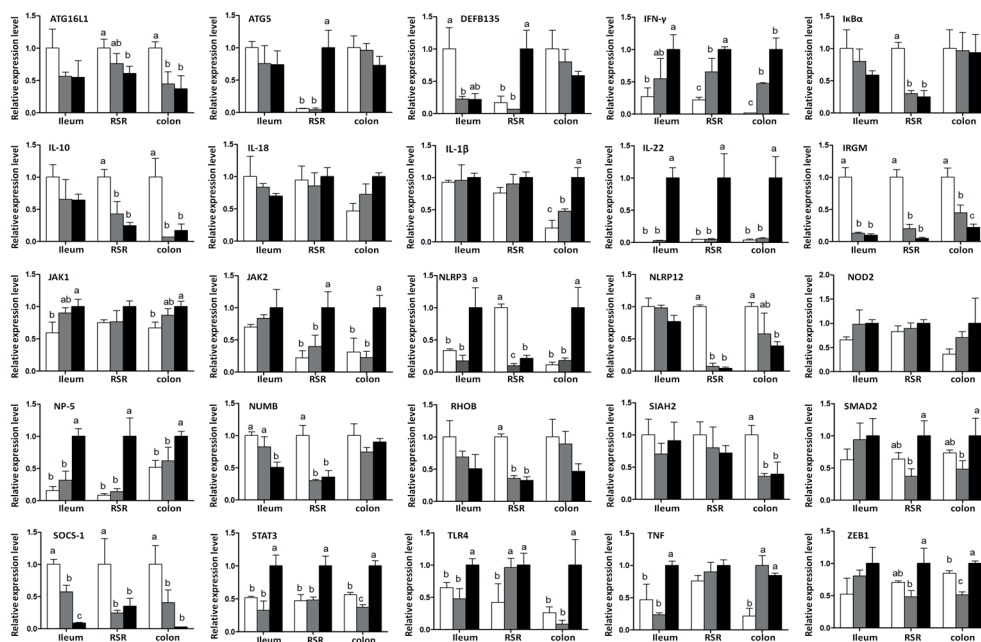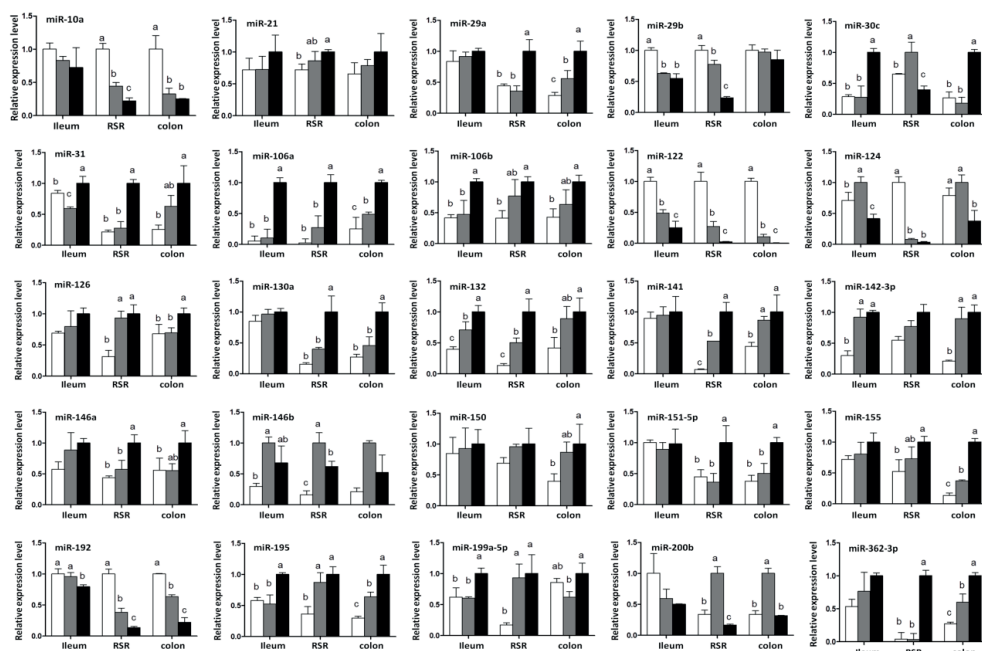

Figure 1: Relative expression levels of 25 genes in different tissues among 3 groups. ☐ Healthy; ▨ Mid; ■ Severe. The highest expression one is set to 1. The expression levels were expressed as −ΔΔCT after normalising with *TBP*. Different lowercase letters show significant difference (*P*<0.05). RSR: rabbit sacculus rotundus.

Figure 2: Relative expression levels of 25 miRNAs in different tissues among 3 groups. ☐ Healthy; ▨ Mid; ■ Severe. The highest expression one is set to 1. Expression levels were expressed as −ΔΔCT after normalising with U6. Different lowercase letters show that the difference is significant (*P*<0.05). RSR: rabbit sacculus rotundus.

Of these 23 genes, we detected 12 genes differentially expressed in ileum, 19 genes differentially expressed in RSR, and 18 genes differentially expressed in colon. In other words, there were 8 significant difference in gene expression among the 3 groups in all tissues, and 10 in gene expression in 2 tissues, and 5 in gene expression in only 1 tissue, and 2 in gene expression in none. The results were also presented via Venn diagrams (Figure 3a). It was shown that 9 genes (*ATG5, IFN-γ, IL-1β, IL-22, JAK1, JAK2, NP-5, TAR4, TNF*) were increased and 8 genes
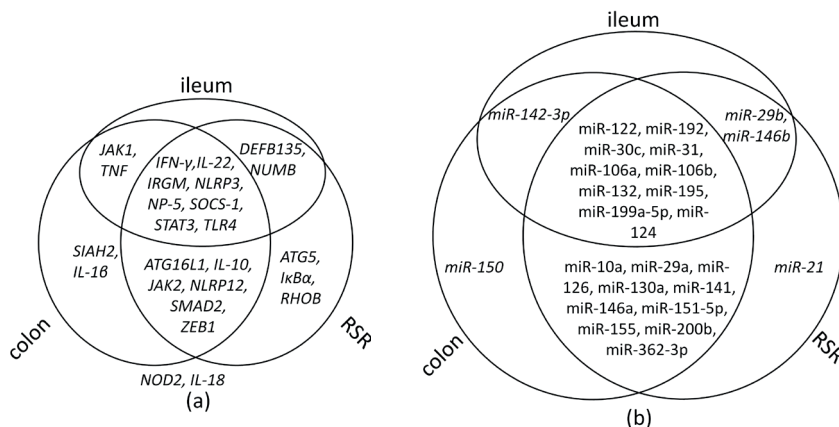

Figure 3: Summary of mRNA and miRNA differentially expressed in different tissues among 3 groups. The Venn diagrams refer to (a) mRNA, (b) miRNA. RSR: rabbit sacculus rotundus.
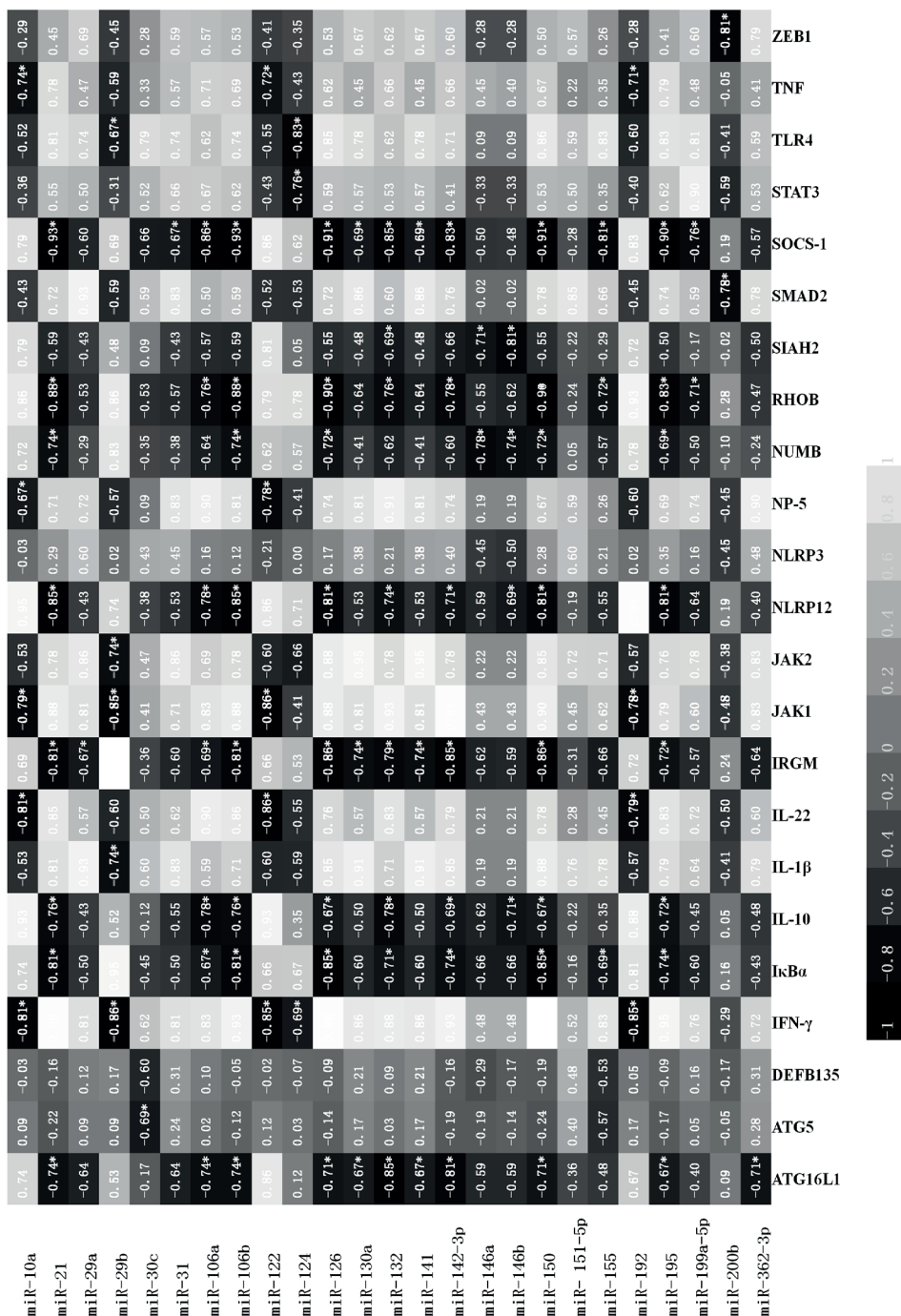
Figure 4: Colour bar of predicted miRNA-mRNA pairs. The 108 significant miRNA-mRNA pairs are in deep black and labelled with an asterisk, and were obtained by anti-correlation and q-value <0.05.

(*ATG16L-1, IκBα, IL-10, IRGM, NLRP12, NUMB, PHOB, SOCS-1*) were decreased along with the severity of NSDD, and 4 genes (*SIAH2, SMAD2, STAT3, ZEB1*) were decreased at first and then increased, and 2 genes (*DEFB135, NLRP3*) were inconsistent in different tissues. These genes are involved in the overall immune system at different subsystems, including 5 mucosal barrier-related genes (*DEFB135, NP-5, RHOB, SMAD2, ZEB1*), 3 autophagy-related genes (*ATG16L1, ATG5, IRGM*), 6 innate immune-related genes (*IκBα, NLRP3, NLRP12, NUMB, SIAH2, TLR4*), and 9 acquired immune-related genes (*IFN-γ, IL-1β, IL-10, IL-22, JAK1, JAK2, SOCS-1, STAT3, TNF*).

Of these 25 miRNAs, 13 miRNAs were differentially expressed in ileum, and 23 miRNAs differentially expressed in RSR, and 22 miRNAs differentially expressed in colon. In other words, 10 miRNAs expressed significant difference in all tissues among 3 groups, and 13 in gene expression in 2 tissues, and 2 in gene expression in only 1 tissue. The results were also presented via Venn diagrams (Figure 3b). It was shown that 18 (miR-21, miR-29a, miR-30c, miR-31, miR-106a, miR-106b, miR-126, miR-130a, miR-132, miR-141, miR-142-3p, miR-146a, miR-150, miR-151-5p, miR-155, miR-195, miR-199a-5p, miR-362-3p) were increased and 4 (miR-10a, miR-29b, miR-122, miR-192) were decreased along with the severity of NSDD, and 2 (miR-146b, miR-200b) were initially increased and then decreased, and 1 (miR-124) was inconsistent in different tissues. These findings prompted us to focus on exploring the relative importance and roles of these miRNAs in regulation of NSDD development.

Statistical significance of miRNA-mRNA pairs

Pearson's correlation analysis was applied to the 25 significantly differentially expressed miRNAs and significantly differentially expressed 23 mRNAs. As miRNAs act as negative regulators, up-regulated miRNAs resulted in down-regulated targeting mRNAs, and vice versa (Bartel, 2004). A total of 108 significant miRNA-mRNA pairs were
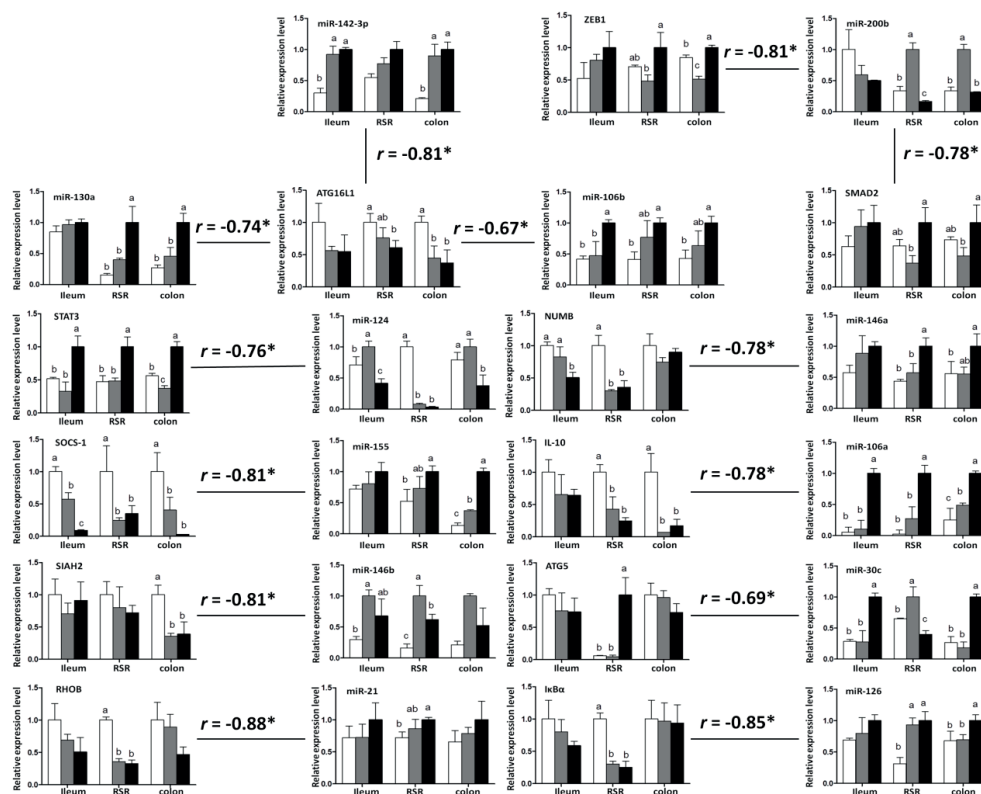

Figure 5: Thirteen significant miRNA-mRNA pairs predicted in the present study. Lines connecting miRNA and mRNA indicated the negative correlation of miRNA and mRNA and significance (* $P<0.05$). RSR: rabbit sacculus rotundus.

obtained by 2 criteria: Pearson's correlation, where less than −0.5 was considered as anti-correlation and a multiple-hypothesis-adjusted *P*-value <0.05 as statistically significant. These 108 predicted miRNA-mRNA pairs contained 24 up-regulated miRNAs targeting down-regulated mRNA pairs and 84 down-regulated miRNAs targeting up-regulated mRNAs pairs (Figure 4). In the up-regulated miRNAs targeting down-regulated mRNA pairs, on average, 1 mRNA was negatively related to 2.3 miRNAs, and 1 miRNAs to 4.0 mRNAs. In the down-regulated miRNAs targeting up-regulated mRNA pairs, on average, one mRNA was negatively related to 10.1 miRNAs, and 1 miRNAs to 4.7 mRNAs. Thirteen pairs predicted in the present study have been previously reported in other studies (Figure 5), leaving the remaining 95 (88.0%) pairs recognised in the current study as potential miRNA-mRNA pairs.

DISCUSSION

NSDD is a major complex intestinal genetic disorder in rabbit, whose precise aetiology and pathogenesis is still not properly understood. Histopathological observation showed that intestinal tissue injury and inflammation were most serious in ileum and colon tissues of the severe NSDD group. And as an important gut-associated lymphoid tissue, RSR plays key roles in the development of an immune response or immune tolerance to the antigens and pathogens in the intestinal tract (Haasz *et al.*, 1974). Recently, several genes have been discovered to be related to the genetic determination of susceptibility to NSDD in rabbit, including *MyD88, TLR4, NOD2, NLRP3, NLRP12, TYK2, JAK1* and *STAT3* (Zhang *et al.*, 2011; Chen *et al.*, 2013a; Liu *et al.*, 2013; Yang *et al.*, 2013b; Zhang *et al.*, 2013; Fu *et al.*, 2014, 2015). It is also proved that several miRNAs play an important role in the pathogenesis of experimental colitis and human IBD. Thus, the expression of 25 immunity genes and 25 miRNAs was analysed in ileum, RSR, and colon tissues from 9 individuals with different disease severity of NSDD using RT-qPCR, which related to mucosal barrier, autophagy, innate immune and acquired immune.

In this study, we found 23 genes and 25 miRNAs differentially expressed among the different degrees of severity of the disease: healthy, mid, and severe. Our findings again suggested the genetic associations between these genetic makers and NSDD in rabbit. Furthermore, 108 possible pairs of miRNA-mRNA with an anti-correlation were identified by Pearson's correlation analysis, which suggested that these pairs of miRNA-mRNA may regulate the development of NSDD. Compared with previous studies, 13 of these miRNA-mRNA pairs were reported in the pathogenesis of IBD (Kalla *et al.*, 2015).

The intestinal mucosal barrier serves as the first line of defence against pathogens, which plays an important role in IBD and its chronicity (Salim and Söderholm, 2011). In this study, 5 genes (*DEFB135, NP-5, RHOB, SMAD2, ZEB1*) and 2 miRNAs (miR-21, miR-200b) related to intestinal mucosal barrier were significantly differentially expressed among groups. In ulcerative colitis (UC) patients, overexpression of miR-21 induced the degradation of *RHOB* mRNA, which led to the depletion of *RHOB* and the impairment of tight junctions in intestinal epithelial cells (Yang *et al.*, 2013a). The over expression of miR-200b enhanced E-cadherin expression through targeting of *ZEB1*, and also reduced Vimentin expression by targeting *SMAD2* (Chen *et al.*, 2013b). Up-regulation of E-cadherin and down-regulation of Vimentin will inhibit epithelial-mesenchymal transition and promote proliferation of intestinal epithelial cells, which is prominently linked to the pathogenesis of IBD.

Autophagy is an essential process important for cell survival and homoeostasis, which has emerged as an important player in disease (Lockshin and Zakeri, 2004). In this study, 3 genes (*ATG16L1, ATG5, IRGM*) and 4 miRNAs (miR-130a, miR-142-3p, miR-106b, miR-30c) related to autophagy were significantly differentially expressed among groups. In CD patients, up-regulation of miR-30c and miR-130a reduced the expression levels of *ATG5* and *ATG16L1* and inhibited autophagy, leading to increased inflammatory response both *in vitro* and *in vivo* (Nguyen *et al.*, 2014). And *ATG16L1* is also a direct target of the miR-106b and miR-93 (Lu *et al.*, 2014).

The innate immune system comprises the cells and mechanisms that recognise and respond to pathogens generically. In this study, 6 genes (*IκBα, NLRP3, NLRP12, NUMB, SIAH2, TLR4*) and 4 miRNAs (miR-122, miR-192, miR-146a, miR-126) related to innate immune system were significantly differentially expressed among groups. Over-expression of miR-126 leads to *NF-κB* activation via down-regulation of *IκBα*, which may contribute to the pathogenesis of human UC (Feng *et al.*, 2012). The miR-146a targeted *NUMB* gene and alleviated the suppression of SHH signalling to amplify inflammatory responses of IBD (Ghorpade *et al.*, 2013; Nata *et al.*, 2013).

The acquired immune system is composed of highly specialised systemic cells and processes, which creates immunological memory after an initial response to a specific pathogen, and leads to an enhanced response to subsequent encounters with that pathogen. In this study, 9 genes (*IFN-γ, IL-1β, IL-10, IL-22, JAK1, JAK2, SOCS-1, STAT3, TNF*) and 2 miRNAs (miR-124, miR-106a) related to innate immune system were significantly differentially expressed among groups. Reduced levels of miR-124 in colon tissues of children with active UC appear to increase expression and activity of *STAT3* gene, which could promote inflammation and pathogenesis of UC in children (Koukos *et al.,* 2013). The miR-106a and miR106b decrease *IL-10* expression by degrading its mRNA, which is a key anti-inflammatory regulator in many model systems (Sharma *et al.,* 2009).

In this paper, we have identified 23 genes and 25 miRNAs differentially expressed in 3 tissues among the different severities of NSDD in rabbit, which constructed a network of 108 miRNA-mRNA pairs using Pearson's correlation analysis. Most of these miRNA-mRNA pairs were hitherto unexplored, and their roles in NSDD biology require further elucidation. Our results give a clue to the potential miRNA-mRNA pairs for NSDD that can further improve understanding of the occurrence and development of NSDD in rabbit.

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