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Full Length Article

Comparative Analysis of Transcriptomes from Synthetic CpG ODN/CpG Free DNA Stimulated Bovine PBMCs

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Abstract

It has been reported that both cytosolic DNA and the single-stranded synthetic phosphorothioate oligodeoxynucleotides containing specific CpG motifs (CpG ODN) could activate the host innate immune system and induce up-regulation of the expression of Type I interferons (IFNs) and pro-inflammatory cytokines, as well as the subsequent induction ISGs. Albeit the similarity in innate immune activation, cytosolic DNA is reported to be recognized and signal through the cGAS-STING-TBK1 axis, while CpG ODN engages with endosomal TLR9. Until recently, few data were available on the transcriptomes of cytosolic DNA or synthetic CpG ODN stimulated PBMCs. In the current study, bovine PBMCs were stimulated with CpG Free DNA or CpG ODN, respectively. RNA-seq technology was employed to study the blood gene expression profiles from the PBMCs treated with each stimulus. DEGs were identified, and the up-regulated DEGs were run through GO and KEGG analysis. The similarities as well as differences between CpG Free DNA or CpG ODN stimulated transcriptomes were compared. Our data provide new information on nucleic acids-drived immune activation and shed light on the use of different forms of nucleic acids as potential adjuvants for bovine vaccines. © 2019 Friends Science Publishers

Keywords: Nucleic acids; Adjuvant; Immune activation; RNA-seq; DEGs

Introduction

Pattern recognition receptors (PRRs) are essential in detecting microbial pathogen-associated molecular patterns (PAMPs) and initiate innate immunity (Janeway and Medzhitov, 2002; Medzhitov, 2009; Hossain and Norazmi, 2013). Several PRRs have been reported to recognize nucleic acids in different sub-cellular compartments (Kawai and Akira, 2010; Paludan and Bowie, 2013; Pachathundikandi *et al.*, 2015; Yoneyama *et al.*, 2015). Among them, Toll-like receptor 9 (TLR9) is located in the endosomes and responds to viral DNA containing specific CpG motifs（CpG ODN）, while cyclic GMP-AMP synthase (cGAS) is recently reported to reside in the cytosol and regarded as a *bona fide* sensor for foreign and self-derived DNA fragments that accumulated in the cytosolic environment (Hemmi *et al.*, 2000; Xiao, 2009; Sun *et al.*, 2013; Ohto *et al.*, 2015).

Upon CpG ODN binding, TLR9 undergoes dimerization, and initiates a signaling cascade through the MYD88 dependent pathway (Xiao, 2009; Mirotti *et al.*, 2017). The cytosolic DNA sensor cGAS recognizes DNA without sequence specificity but in a length-dependent manner (Luecke *et al.*, 2017) and catalyzes the production of a second messenger-cGAMP, which activates the STING/TBK1/IRF3 pathway (Ablasser *et al.*, 2013; Chen *et al.*, 2016). Although both TLR9 and cGAS activation can each lead to the up-regulation of pro-inflammatory cytokines and Type I IFNs, as well as the subsequent induction of many interferon-stimulated genes (ISGs), differences between the transcriptomes of TLR9 or cGAS-mediated cytokine expression profiles are rarely reported. Since CpG ODN mediated TLR9 activation and cytosolic DNA stimulated cGAS activation signal *via* separate transduction pathways, it is hypothesized that differences could exist between the resultant cytokine profiles after treatment of immune cells with each stimulus.

In the current research, bovine peripheral blood mononuclear cells (PBMCs) were treated with CpG Free DNA or CpG ODN, respectively. The gene expression profiles in the PBMCs triggered by different stimuli were investigated *via* RNA-seq using the next-generation sequencing (NGS) technology. The differences between the CpG Free DNA or CpG ODN stimulated transcriptomes, in comparison to the Mock-treated group, were analyzed.

Materials and Methods

Animals and Ethics Statement

A total number of 3 twelve-month-old cows used for blood

collection were housed in appropriate containment facilities. All animals have been tested free of infection by bovine tuberculosis and brucellosis, and vaccinated against Infectious Bovine Rhinotracheitis, Bovine Viral Diarrhea and Foot-and-mouth Disease. During the whole experiment period, no clinical signs of infection have been observed among the animals. Experimental procedure for blood collection in this research was approved by the Animal Ethics Committee of Institute of Animal Science (IAS).

PBMC Isolation and Treatment

Bovine PBMCs were prepared by density-gradient centrifugation over Ficoll (Hu *et al.*, 2016). The viability of the PBMCs was checked by trypan blue staining and cell viability was calculated as＞95%. Isolated PBMCs were counted and allocated into 6-well plates at 1×10^7 cells per well in RPMI-1640 medium supplemented with 10% FBS (Hyclone). Cells were incubated in 5% CO_2 cell culture incubator under standard conditions. For each blood sample, the collected PBMCs were stimulated with either 5 μg/ mL CpG Free DNA in Group 1 (pCpGfree, InvivoGen) or 5 μg/mL phosphorothioate CpG ODN in Group 2 (ODN 2007, TLR9 agonist: 5'- tcgtcgttgtcgttttgtcgtt -3') for 12 h. Cells from the Control Group were mock-treated with PBS. Each stimulation was performed in duplicate (two wells).

RNA Extraction, cDNA Libraries Preparation and RNA-Sequencing

Whole blood RNA from each group was extracted using a Mini-BEST Universal RNA Extraction Kit (Liuhetong Co, Dalian, China). The RNA samples with the same stimulus were pooled together for subsequent transcriptome analysis. The cDNA libraries were generated using a NEBNext® Ultra™ RNA Library Prep Illumina Kit for (New England Biolabs Inc.). Briefly, mRNA was isolated from the high-quality total RNA samples using NEBNext Poly(A) mRNA Magnetic Isolation Module, and further fragmented into about 200bp. First-strand cDNA synthesis was performed using NEBNext® Multiplex Oligos for Illumina® (Promega, Madison, WI, USA), and then second-strand cDNA was synthesized. After purification with the Agencourt AMPure XP beads (Beckman Coulter, Inc.), the cDNA fragments were end-repaired and ligated to the Solexa adaptor. The desired size of adaptor-ligated fragment was selected by AMPure XP beads. The suitable cDNA fragments were PCR-amplified to construct cDNA libraries. Finally, paired-end sequencing was carried out using HiSeq X Ten System (Illumina Inc.). Sequencing was performed at ori-gene Ltd. (Beijing, China).

Bioinformatics Analysis

The raw reads were processed *via* cutadapt (http://cutadapt.readthedocs.org/). Low quality sequence reads from both ends, adaptors, sequences containing unknown nucleotides (reads with ambiguous "N" bases) or sequence length less than 60bp were all removed. Quality assessment before and after raw data processing was performed using FastQC tool. All clean reads were aligned to the reference genome (ftp://ftp.ensembl.org/pub/release-87/fasta/bos_taurus/dna/Bos_taurus.UMD3.1.dna.toplevel.fa) using the TopHat2 software to obtain the mapped reads for further bioinformatics analysis. The mapped reads were run through Cufflinks (http://cufflinks.cbcb.umd.edu/Version 2.2.1) for the identification of new transcripts and novel genes.

Differentially Expressed Genes (DEGs)

DEGs among mock-treated, CpG Free DNA or CpG ODN stimulated groups were analyzed using cuffdiff (http://cufflinks.cbcb.umd.edu/). In the current study, total mapping reads≥10, $|\log2(FC)|\geq1$, $P \leq 0.05$ and $Q \leq 0.05$ (FDR≤1%) were set as the thresholds of significant differences in gene expression between compared groups (Trapnell *et al.*, 2013).

Functional Annotation

The differentially up-regulated DEGs were run through the databases of GO and KEGG for enrichment analysis. The major biological functions of DEGs were annotated *via* GO enrichment analysis (http://www.geneontology.org/). DEGs-associated pathways were analyzed *via* KEGG database (http://www.genome.jp/kegg/).

qRT-PCR

For technical validation of the gene expression data, qRT-PCR was carried out for 6 genes: ISG15, MX1, OAS1Y and PKP2 (significantly up-regulated), ASRGL1 and XLOC_007455 (significantly down-regulated). qRT-PCR was carried out using the corresponding pooled RNA sample as template on Roche LightCycler® 96 using Genstar SYER FAST qPCR Kit Master Mix (2×). The primers used for qRT-PCR were given in Table 1. All reactions were run in triplicates. Based on previous report (Robinson *et al.*, 2007), the house-keeping gene encoding bovine β-actin was adopted for normalization of target genes of interest. The relative expression of each sample was determined by the $2^{-\Delta\Delta Ct}$ method. Differences in mRNA levels between the CpG Free DNA/CpG ODN stimulated and mock-treated bovine PBMCs were statistically analyzed using *t*-tests.

Results

Overview of the Sequencing Data

A total amount of 9.72 gigabases (Gb) bases was obtained from the three RNA-seq libraries. After filtering the Raw Data to remove low-quality reads, 8.78 Gb bases was

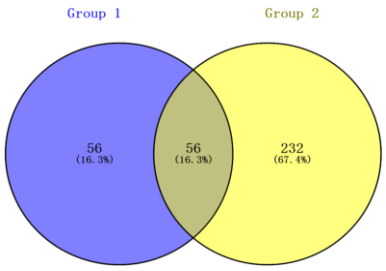

Fig. 1: Intersecting Venn diagram showing the abundance of DEGs. The number of genes differentially up-regulated in the CpG Free DNA (Group 1) /CpG ODN (Group 2) stimulated bovine PBMCs, compared to the mock-treated group, were run through the VENNY 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The numbers in overlapping area represent the shared genes of differential up-expression among the two stimulated groups

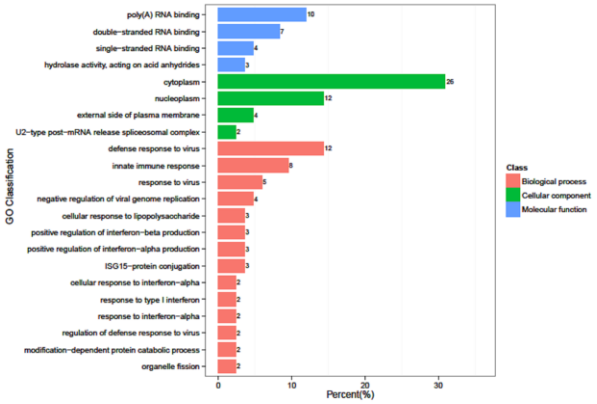

Fig. 2: GO Enrichment Analysis of differentially up-regulated DEGs from the CpG Free DNA-stimulated bovine PBMCs. The DEGs are classified into three categories: cellular component, molecular function and biological process. The percentage of genes in each category and the number of genes are shown above

regarded as Clean Data, and used for later reference genome mapping. A high percentage of the reads (88.62 to 89.31%) were mapped to the cow reference genome. Among them, 76.30–86.28% was uniquely mapped to the cow reference genome. Among the total 17652 genes analyzed, 12315 genes (69.8%) have been annotated in the reference genome, while 5337 genes (30.2%) have been identified as novel genes.

Differential Gene Expression Analysis

Bioinformatics analysis showed that 124 and 355 genes were found to be differentially expressed in the CpG Free DNA (Group 1) /CpG ODN (Group 2) stimulated groups, respectively, compared to the mock-treated group. Among them, 112 and 288 genes were up-regulated in Groups 1 and 2, respectively. Group 1 and Group 2 shared in common 56 genes (16.3%) in the up-regulated DEGs (Fig. 1), while 4 genes were shared among the down-regulated ones.

GO Enrichment Analysis of Up-regulated DEGs

The biological functions of the differentially up-regulated DEGs from Groups 1 and 2, compared to the mock-treated group, were run through GO enrichment analysis. 22 and 46 significantly enriched GO terms were identified for Groups 1 and 2, respectively.

In Group 1, our results showed that the major functional terms of DEGs in biological process included defense response to virus, innate immune response, response to virus, negative regulation of viral genome replication, ISG15-protein conjugation, positive regulation of interferon-alpha production, positive regulation of interferon-beta production and cellular response to lipopolysaccharide, etc., the major terms in cellular component included cytoplasm, nucleoplasm and external side of plasma membrane, etc.; the major terms in molecular function included poly(A) RNA binding, double-stranded RNA binding, single-stranded RNA binding and hydrolase activity (Fig. 2).

In Group 2, GO analysis showed that the major functional terms of DEGs in biological process included positive regulation of transcription from RNA polymerase II promoter, defense response to virus, chemokine-mediated pathway, cellular response to interleukin-1, cellular response to tumor necrosis factor and ubiquitin-dependent protein catabolic process, etc.; the major terms in cellular component included nucleus, nucleoplasm, centrosome and chromatin, etc., the major terms in molecular function included ATP binding, poly(A) RNA binding, zinc ion binding and nucleic acid binding, etc. (Fig. 3).

Interestingly, GO analysis of the differentially up-regulated DEGs in Group 1, compared to Group 2, showed that the major functional terms of DEGs in biological process included defense response to virus, negative regulation of viral genome replication, response to type I interferon and response to virus, while no enrichment terms were obtained for the cellular component or molecular function. Compared to Group 1, analysis of the differentially up-regulated DEGs in Group 2 showed that no enrichment terms were obtained.

KEGG Pathway Enrichment Analysis of up-regulated DEGs

During KEGG analysis, several innate immune-relevant pathways involving the up-regulated DEGs from Groups 1 and 2 (compared to the mock-treated group) were found to be significantly enriched. In Group 1, the enriched pathways included the cytosolic DNA-sensing pathway, the RIG-I-like receptor pathway, and pathways related to influenza A, measles, herpes simplex infection, hepatitis C, hepatitis B, and transcriptional mis-regulation in cancer (Table 2).

In Group 2 the enriched pathways included cytokine-cytokine receptor interaction, chemokine pathway, Toll-like receptor pathway, apoptosis, NF-kappa B pathway, TNF pathway, NOD-like receptor pathway, Jak-STAT pathway, cytosolic DNA-sensing pathway, RIG-I-like receptor pathway

Table 1: Genes and their primer sequences used for validation of the RNA-sequencing data. In total, six genes were selected for Quantitative PCR verification, and β-actin was chosen as an internal control for the normalization of results

Genes	Primer sequences	Product length (bp)	Accession Numbers
β-actin	Forward: TGGACTTCGAGCAGGAGAT	151	NM_174366
	Reverse: CGTCACACTTCATGATGAAA		
ISG15	Forward: CATCCTGGTGAGGAACGACA	151	NM_174366
	Reverse: ATCATCCATGGGCCTCCCTT		
MX1	Forward: AGAGCCCTCCACAGATGAGA	108	NM_173940
	Reverse: CGGAGCACGAAGAACTGGAT		
OAS1Y	Forward: AGTGACCTGATGTTCCAGGC	130	NM_001040606
	Reverse: ACTCTGGCGTTCAGAGGATG		
PKP2	Forward: TCCATGGTCCTTGAATCCAGA	118	NM_001083729
	Reverse: CCATGCACCATTAGCCCTGA		
ASRGL1	Forward: CGCAGGTTGTGGATCTGTCT	161	NM_001077035
	Reverse: AGCAGTGAGGTGTCTTGTCC		
XLOC_007455	Forward: AGATCCAGGGAAGTCCGAGA	130	—
	Reverse: CTTTTACAAGTGCAGCCTGGG		

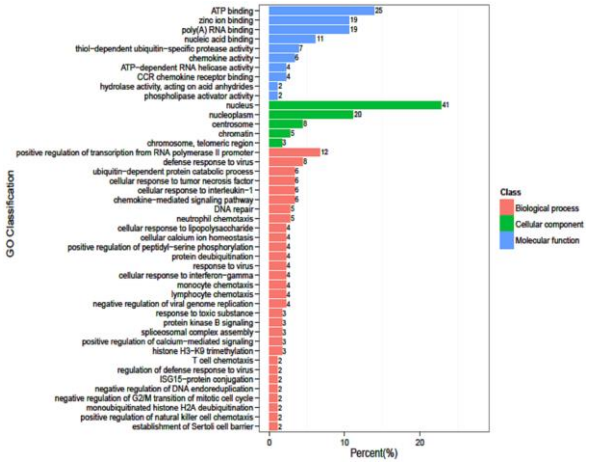

Fig. 3: GO Enrichment Analysis of differentially up-regulated DEGs from the CpG ODN-stimulated bovine PBMCs. The DEGs are classified into three categories: cellular component, molecular function，and biological process. The percentage of genes in each category and the number of genes are shown above

and pathways related to influenza A, salmonella infection, measles, transcriptional misregulation in cancer, and rheumatoid arthritis (Table 3). However, when the up-regulated DEGs between Group 1 and Group 2 were compared (16 DEGs in Group 1 and 33 in Group 2), no enriched pathways involving innate immunity were determined.

qRT-PCR Validation Study

qRT-PCR was carried out for the verification of gene expression data obtained *via* RNA-seq. A total of six genes, four differentially up-regulated and two differentially down-regulated in both Groups 1 and 2 (compared to the mock-treated Group), were chosen for analysis. Fold changes of the target genes in RNA-seq and qRT-PCR were compared. The qRT-PCR results were generally consistent with the RNA-seq results, confirming the reliability of the results from RNA-seq (Table 4 and 5).

Discussion

The innate immune system constitutes the “front line” in combating microbial infection, using various PRRs to detect microbial-derived PAMPs (Medzhitov, 2007). Toll-like receptors are members of the transmembrane PRRs family, and are among the most studied PRRs. Up to now, 10 and 13 members of the Toll-Like Receptor family has been identified in human and mouse, respectively (Vacchelli *et al.*, 2013; Vidya *et al.*, 2018). Among them, TLR9 is located on intracellular vesicles and reported to recognize CpG DNA (Hemmi *et al.*, 2000). CpG ODN-induced gene expression profiles in pigs have been recently reported, in which a large number of immune-relevant DEGs are significantly up-regulated (Hu *et al.*, 2016). This is in correspondence with our results, as CpG ODN-stimulated bovine PBMCs also witnessed a wide range of innate immune activation.

On the other hand, multiple cytosolic DNA sensors have been recently discovered, including DAI, STING, IFI16 and cGAS etc. (Ishikawa and Barber, 2008; Kaiser *et al.*, 2008; Unterholzner *et al.*, 2010; Sun *et al.*, 2013). The cGAS-STING-IRF3 signaling axis has been regarded as the key signaling pathway involved in cytosolic DNA sensing (Tao *et al.*, 2016; Xia *et al.*, 2016). Recent study also highlighted the importance of cGAS-mediated gene expression in anti-viral immune response (Schoggins *et al.*, 2014).

As TLR9-and cGAS- mediated immune activation signal through different adaptor molecules and separate transduction pathways, we hypothesized that differences may exist between their stimulated cytokine profiles. During our study, bovine PBMCs were stimulated with either CpG Free DNA or phosphorothioate CpG ODN and the transcriptomes from the PBMCs were analyzed *via* RNA-seq to identify global gene expression changes. Meanwhile, the gene expression profiles between CpG Free DNA/CpG ODN stimulated transcriptomes were compared.

As identified in our current research, many ISGs (including ISG15, IFIT5, MX1, etc.) have been up-regulated in the bovine PBMCs during CpG ODN/CpG Free DNA treatment. Hence, our results re-confirm that the two forms of nucleic acid are potent stimulators of innate immunity, which may have major contribution to the clearance of infection and be the key factor influencing the quality of subsequent adaptive immune response (Mosca *et al.*, 2008;

Table 2: The major enriched KEGG pathways analysis from CpG Free DNA stimulated bovine PBMCs. The significantly up-regulated DEGs from CpG Free DNA stimulated bovine PBMCs (compared to the Control Group) were analyzed *via* KEGG database. The DEGs-associated immune relevant pathways (with *P* Value ≤ 0.05) were listed

Pathways	btaID	Genes	*P* Value
Influenza A	bta05164	RSAD2, OAS1X, CXCL10, TMPRSS2, DDX58, MX1, TNFSF10, OAS1Y, IFIH1, IL6, IRF7, EIF2AK2	1.15E-10
Measles	bta05162	OAS1X, DDX58, MX1, TNFSF10, OAS1Y, IFIH1, IL6, IRF7, EIF2AK2	1.34E-07
Cytosolic DNA-sensing pathway	bta04623	MB21D1, ZBP1, CXCL10, DDX58, IL6, IRF7	6.63E-06
RIG-I-like receptor pathway	bta04622	CXCL10, DDX58, LGP2, ISG15, IFIH1, IRF7	1.96E-05
Herpes simplex infection	bta05168	OAS1X, DDX58, OAS1Y, IFIH1, IL6, IRF7, EIF2AK2	1.71E-04
Hepatitis C	bta05160	OAS1X, DDX58, OAS1Y, IRF7, EIF2AK2	2.79E-03
Hepatitis B	bta05161	DDX58, IFIH1, IL6, IRF7	2.80E-02
Transcriptional misregulation in cancer	bta05202	TMPRSS2, REL, IL6, CCNT2	4.22E-02

Table 3: The major enriched KEGG pathways analysis from CpG ODN stimulated bovine PBMCs. The significantly up-regulated DEGs from CpG ODN stimulated bovine PBMCs (compared to the Control Group) were analyzed *via* KEGG database. The DEGs-associated immune relevant pathways (with *P* Value ≤ 0.05) were listed

Pathways	btaID	Genes	*P* Value
Influenza A	bta05164	RSAD2, CXCL10, MX1, CXCL8, TNFSF10, IFIH1, IL6, EIF2AK2, PIK3CA	3.98E-05
Cytokine-cytokine receptor interaction	bta04060	CSF3, CCL4, CXCL10, CCL3, CXCL8, CCL20, TNFSF10, CCL8, IL6	3.66E-04
Chemokine pathway	bta04062	CCL4, CXCL10, CCL3, CXCL8, CCL20, CCL8, ROCK1, PIK3CA	4.33E-04
Toll-like receptor pathway	bta04620	CCL4, CXCL10, CCL3 ,CXCL8, IL6, PIK3CA	1.09E-03
Apoptosis	bta04210	XIAP ,ATM, TNFSF10, PIK3CA, BIRC3	1.15E-03
Salmonella infection	bta05132	CCL4, CCL3, CXCL8, IL6, ROCK1	3.39E-03
Measles	bta05162	MX1, TNFSF10, IFIH1, IL6, EIF2AK2, PIK3CA	3.87E-03
NF-kappa B pathway	bta04064	CCL4, XIAP, CXCL8, BIRC3, ATM	4.72E-03
TNF pathway	bta04668	PIK3CA, IL6, BIRC3, CXCL10, CCL20	8.62E-03
Transcriptional misregulation in cancer	bta05202	CCNT2, IL6, CXCL8, JMJD1C, ATM, ELK4	9.60E-03
Cytosolic DNA-sensing pathway	bta04623	CCL4, IL6, MB21D1, CXCL10	1.06E-02
RIG-I-like receptor pathway	bta04622	IFIH1, CXCL8, ISG15, CXCL10	1.92E-02
Rheumatoid arthritis	bta05323	IL6, CCL3 ,CXCL8, CCL20	3.43E-02

Table 4: RNA samples from CpG free DNA -stimulated and Mock-treated bovine PBMCs were run in parallel through qPCR. Fold changes of the target genes in qRT-PCR analysis and RNA-seq were compared. Results showed that the expression patterns of the selected genes were generally consistent with the RNA-seq results, confirming the reliability of the data from RNA-seq

Gene ID	Gene	Quantitative PCR validation of RNA-seq			
		Transcriptome		qPCR	
		Fold change[a]	P value[b]	Fold change[a]	P value[b]
ENSBTAG00000014707	ISG15	22.10	5.00E-05***	61.11	1.46E-08***
ENSBTAG00000030913	MX1	5.99	5.00E-05***	10.74	1.06E-03***
ENSBTAG00000039861	OAS1Y	6.08	5.00E-05***	16.35	8.62E-06***
ENSBTAG00000002651	PKP2	2.17	5.00E-05***	3.15	7.64E-06***
ENSBTAG00000006910	ASRGL1	0.46	4.00E-04***	0.65	5.65E-03**
XLOC-007455	__	0.43	5.00E-05***	0.70	3.36E-04***

[a] Fold change in gene expression from CpG free DNA-stimulated bovine PBMCs relative to the Mock-treated group
[b] *P < 0.05, **P < 0.01, ***P < 0.001

Table 5: RNA samples from CpG ODN-stimulated and Mock-treated bovine PBMCs were run in parallel through qPCR. Fold changes of the target genes in qRT-PCR analysis and RNA-seq were compared. Results showed that the expression patterns of the selected genes were generally consistent with the RNA-seq results, confirming the reliability of the data from RNA-seq

Gene ID	Gene	Quantitative PCR validation of RNA-seq			
		Transcriptome		qPCR	
		Fold change[a]	P value[b]	Fold change[a]	P value[b]
ENSBTAG00000014707	ISG15	7.43	5.00E-05***	11.88	2.84E-06***
ENSBTAG00000030913	MX1	2.23	2.00E-04***	3.19	1.52E-02*
ENSBTAG00000039861	OAS1Y	2.29	9.00E-04***	3.84	3.55E-05***
ENSBTAG00000002651	PKP2	2.21	5.00E-05***	2.03	1.67E-02*
ENSBTAG00000006910	ASRGL1	0.33	5.00E-05***	0.44	7.22E-04***
XLOC-007455	__	0.24	5.00E-05***	0.34	1.13E-05***

[a] Fold change in gene expression from CpG ODN-stimulated bovine PBMCs relative to the Mock-treated group
[b] *P < 0.05, **P < 0.01, ***P < 0.001

Schoggins *et al*., 2014; Reis *et al.*, 2017). However, despite the common effects in innate immune activation by CpG ODN or CpG Free DNA, clear differences have been observed in the transcriptomes from CpG ODN/CpG Free DNA stimulated bovine PBMCs. CpG ODN showed a broader stimulation spectrum as indicated in the number of up-regulated DEGs and enriched pathways. CpG Free DNA simulated an innate immune response more likely mimic the cytosolic viral infection, as indicated by the enrichment GO analysis compared to Group 2. Taken together, we conclude that both CpG ODN and CpG Free DNA are potent simulators of innate immune response and they could be used as adjuvants for bovine vaccines in which a Th1-biased immune response is required (Snider *et al.*, 2014; Hagan *et*

al., 2015). Hence, this study provided useful information for the development of novel immunoadjuvants based on PAMPs.

Conclusion

In this study, next-generation sequencing data and subsequent analysis provide comprehensive transcriptome information for CpG Free DNA or CpG ODN stimulated bovine PBMCs. During GO Enrichment and KEGG Pathway Enrichment Analysis, both CpG Free DNA and CpG ODN stimulated bovine PBMCs showed the activation of several innate immune-relevant pathways, compared to the mock-treated group. Yet differences have been identified in the transcriptomes from CpG ODN or CpG Free DNA stimulated bovine PBMCs, possibly due to the different memberane-bound or cytosolic PRRs for each stimulus.

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