RNA silencing-targeted transcriptome of porcine alveolar macrophages upon infection with porcine reproductive and respiratory syndrome viruses (PRRSV) of different virulence

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To cite this version:

Sophie Dhorne-Pollet, Patricia Renson, Florence Jaffrezic, Guillemette Marot, Marco M. Moroldo, et al.. RNA silencing-targeted transcriptome of porcine alveolar macrophages upon infection with porcine reproductive and respiratory syndrome viruses (PRRSV) of different virulence. 35. Conference of the International Society for Animal Genetics (ISAG), Jul 2016, Salt Lake City, Utah, United States. 197 p. hal-02740229

HAL Id: hal-02740229

https://hal.inrae.fr/hal-02740229

Submitted on 2 Jun 2020

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Residual feed intake (RFI) is described as the difference between an individual's actual feed intake and its predicted feed requirements for maintenance and growth. The objective of this study was to investigate the molecular mechanisms contributing to differences in RFI. cDNA obtained from Longissimus thoracis et lumborum (LTL) muscle of 20 commercial line Maxgro x (Landrace x Large White) gilts from low and high RFI groups was hybridized on Affymetrix Snowball Array. Samples were RMA normalized, and probe sets with a low standard deviation ($s \leq 0.23$) were discarded. A further analysis involved filtering by both control probe sets and means (means ≤ 2.5 were rejected). Mixed-model analysis was implemented, and pathway analysis was conducted. A total of 30,992 probe-sets remained after filtering, and 423 genes were found to be at least 1.5-fold differentially expressed. The most altered genes were AP2M1 (2.37; highRFI < lowRFI) and NCOA2 (3.32; highRFI > lowRFI), respectively. The most significant molecular and cellular functions of differentially expressed (DE) genes in relation to RFI were "accumulation of fatty acid" and "accumulation of lipid." The most significant canonical pathways DE in relation to RFI were "TR/RXR activation" and "PEDF signaling." To validate the microarray, a set of reference genes was selected (B2M, RPL10, RPS11). Out of 10 DE genes, mRNA abundance of nine transcripts (ACACA, ACSL1, BCL2, CAPN6, JMJD1C, NCOA1, RHOA, WIPF1 and PPARG) showed a numerical change in the same direction when compared to the microarray. In conclusion, a number of pathways were altered in relation to RFI groups in LTL muscle, and many of the addressed biological processes could be broadly classified into three categories: accumulation of fatty acids, adhesion of connective tissue and apoptosis. Thus, those transcripts are potential candidate genes for improved efficiency.

Key Words: residual feed intake, gene expression, pig

P3025 RNA depletion for highly abundant transcripts in bovine mammary gland improves the sensitivity of RNaseq analysis. R. Weikard,* C. Kühn (Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany)

Deep RNA sequencing (RNaseq) generates the comprehensive transcriptomic landscape of cells and tissues and has opened a new horizon for understanding global gene expression. In the transcriptome of the mammary gland of lactating dairy cows, genes encoding for milk proteins are highly abundant, which can impair prevent the detection of lowly expressed transcripts at a given level of sequence depth. The aim of this study was to develop a cost-efficient, bovine-specific procedure to decrease the proportion of highly expressed transcripts in mRNA from mammary gland for improving the sensitivity to discover transcripts with marginal expression levels. Therefore, antisense oligonucleotides targeting genes of the casein cluster (CSN1S1, CSN1S2, CSN2 and CSN3), α-lactalbumin (LALBA) and β-lactoglobulin (LGB) near their polyA tail were hybridized to total RNA isolated from mammary gland of lactating dairy cows. Digestion with RNase H followed by poly(A+) mRNA selection leads to a RNA depletion of the targeted genes encoding milk protein genes (deadenylated) in the mRNA pool. The effect of this RNA pre-treatment before RNaseq was monitored by comparative expression analysis of depleted and nondepleted RNA samples using reverse-transcription qPCR (RT-qPCR). The results showed that the mRNA expression level of targeted milk protein genes was reduced by 30% to 90% in the depleted samples depending on the specific gene targeted. Exemplarily, RNaseq libraries were prepared from depleted and nondepleted RNA from the same animals and subjected to paired-end mRNaseq analysis on the HiSeq 2500 Sequencing System (Illumina). The results obtained by RT-qPCR were also reflected by whole transcriptome analysis. In response to RNase H-mediated RNA depletion, the ratio of reads mapping to the targeted milk protein genes relative to the whole number of reads decreased from about 60% in the nondepleted sample to 30% in the depleted sample. Furthermore, the sensitivity for discovering transcripts with low expression levels was improved. To further optimize the efficiency of the RNase H-mediated RNA depletion, experimental conditions could be modified — for instance, the sequences or ratios of oligonucleotides used for depletion of targeted milk protein genes.

Key Words: RNaseq, mammary gland, RNA depletion, cattle

P3026 RNA silencing-targeted transcriptome of porcine alveolar macrophages on infection with porcine respiratory and reproductive syndrome viruses (PRRSV) of different virulence. S. Pollet (GABI, INRA, AgroParisTech, Universite Paris Saclay, 78350 Jouy en Josas, France), P. Renson (ANSES, Unité Virologie Immunologie Porcines, 22440 Ploufragan, France), F. Jaffrezic (GABI, INRA, AgroParisTech, Universite Paris Saclay, 78350 Jouy en Josas, France), G. Marot

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Porcine reproductive and respiratory syndrome (PRRS) is a major swine disease caused by PRRSV, a positive-sense ssRNA virus present worldwide with a wide range of strains with different virulence and pathogenicity. RNA silencing is a crucial cell component of host virus interactions. Some host miRNAs are known to modulate fundamental components of the host immune response to PRRSV and may directly interact with the expressed PRRSV genome and/or be altered by PRRSV for immunosuppression and/or immunoevasion mechanisms. Here we characterized the pool of host genes modulated by RNA silencing following in vitro infection of porcine alveolar macrophages with two European PRRSV strains (Finistere and Lena). We used four biological replicates and multiplicity of infection of 2, and collected cells at 7 h p.i. and 10 h p.i. Total cell and RISC (RNA-Induced Silencing Complex)-bound immunoprecipitated transcripts were profiled using a custom Agilent 8x60K microarray enriched for host immunity related genes and the expressed genome of each PRRSV strain. Analyses of differentially expressed transcripts in total cell RNA were performed using the Limma R package, while the Anota R package was used for the relative enrichment analysis of RISC-bound vs. total cell transcriptome. As expected, Lena was highly virulent compared to Finistere. Major differences were found in virus titers and total cell RNA expression, with principal component analysis clearly grouping each virus/time and controls. The number of differentially expressed transcripts at 7 h p.i. and 10 h p.i. ($p < 0.05$) increased markedly, from 535 to 2530 for Finistere and from 11,850 to 53,400 for Lena. A completely different pattern was found in the RISC. Between 7 h and 10 h p.i., the number of relatively RISC-enriched host transcripts in Finistere-infected cells was very high at 7 h p.i. (2880) and sharply decreased at 10 h p.i. (250), indicating that several host genes were targeted by RNA silencing mechanisms but only at the early stage of PRRSV infection. Conversely, no significant host transcripts were found to be enriched in RISC for the Lena-infected cells either at 7 h or 10 h p.i. This suggested that at 7 h p.i., the infection was already too progressed to detect any effect, but also that the modulatory effects of the RNA silencing pathways were rapidly overpowered by high virulence strains. Finally, no PRRSV transcripts were found enriched in RISC. Analyses are currently in progress to characterize the gene pathway components of RISC-enriched genes and their predicted targeting by host miRNAs.

Key Words: RISC, miRNA

P3027 Bioactivity of colostrum and milk exosomes containing microrna from cows genetically selected as high, average and low immune responders based on their estimated breeding values. M. Ross* (Department of Pathobiology, University of Guelph, Guelph, ON, Canada), H. Atalla (Department of Pathobiology, University of Guelph, Guelph, ON, Canada; Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada), B. Mallard (Department of Pathobiology, University of Guelph, Guelph, ON, Canada; Department of Animal Biosciences, Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada)

Bovine milk contains bioactive components with immune-regulatory potential in humans. Expression of milk bioactive components is often controlled by microRNA (miRNA). Colostrum and milk miRNAs are enclosed in exosomes, conferring their protection from degradation and potentially promoting uptake by recipient cells. While dairy cows classified as high immune responders (HIR) have improved colostrum and milk quality compared with average (A) and low (L) responders, the bioactivity of colostrum and milk exosome-derived miRNA at the human intestinal epithelial barrier remains to be explored. Therefore, the purpose of this study is to evaluate the functional role of milk exosomes at the intestinal epithelial interface using healthy and cancerous human intestinal epithelial cells. Exosomes were isolated by differential ultracentrifugation from the colostrum and milk of cattle genetically selected as L, A or HIRs based on their estimated breeding values. Exosomes were viewed by electron microscopy and confirmed by immunoglod labeling, ELISA ExoEL kit and Western blot analysis for the presence of common exosomal-proteins (CD9, CD63, CD81, and Hsp70). Quantification of exosomal protein was conducted by BCA protein assay. Exosome surface markers are more abundantly expressed in colostrum exosome isolates across all immune response groups compared with milk. Specifically, expression of colostrum exosome markers is higher in A and HIR exosome isolates, compared with L responders. To assess bioactivity, exosomes