557. Identification of differentially expressed transcription factors in Brangus skin infested with *Rhipicephalus australis*

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Abstract

Tick resistance is a desirable trait in beef cattle to minimise the incidence of cattle tick outbreaks and production loss in tropical and subtropical regions. Identification of genetic determinants of this trait will be beneficial for improved breeding programs. Using RNA-Sequencing, this study investigated gene expression data from skin of tick-infested Brangus cattle with high (n=5) and low (n=6) resistance. There were 229 significant differentially expressed genes (DEGs; FDR<0.05), of which 212 were target genes for 1,866 transcription factors expressed in skin. Regulatory impact factor analysis showed 158 significant TFs (P<0.05) of which *GRHL3*, and *DTX1* were also DEGs in the experiment. Biological pathway enrichment showed the significant TFs and DEGs feature in pathways such as response to infection, transcriptional regulation, and Notch signalling. These results highlight the application of bioinformatic approaches in the identification of key transcriptional factor that are potential drivers of differential expression of host resistance trait in cattle. Thus, these candidate genes could be further explored in gene selection programs to breed for tick resistance in crossbred cattle.

Introduction

Cattle tick infestations negatively affect animal health and cattle production in tropical and subtropical regions worldwide. Tick resistance in cattle is an attractive trait to include in breeding programs as a sustainable solution to control tick outbreaks. However, the selection of tick resistant cattle is still difficult in the field due to reliance on phenotypic assessment by tick scoring methods which are not widely standardised. The application of omics technologies, such as RNA-seq, offer an opportunity to investigate transcripts and regulatory elements associated to this trait. In this context, this paper presents data on skin gene expression from Brangus cattle of divergent host resistance phenotypes (high and low) following 12 weeks of artificial tick challenge with the cattle tick *Rhipicephalus australis*. We hypothesize it is possible to identify the key regulators among skin expressed transcription factors through bioinformatic analysis.

Materials & methods

Animals. 30 Brangus steers (~9 months old) without previous exposure to ticks were recruited for this study conducted under animal ethics approval (QAAFI/469/18). The animals were exposed to artificial infestation with approximately 10,000 tick larvae (*R. australis*) over 12 weeks, during which animals were ranked for their resistance to infestation and skin biopsies were collected on day 0 and week 12 of the trial. The number of developing adult ticks after an infestation cycle (21 days) was estimated with a tick scoring scale from 1 (<50 ticks = High resistance, HR) to 5 (>300 ticks = Low resistance, LR). Animals were ranked according to their mean tick score collected from week 8 to 15 of the trial and the top 6 and bottom 6 animals were selected for skin RNA-Sequencing experiments.

RNA isolation from skin biopsies and sequencing. RNA was extracted from frozen skin preserved in RNAlater reagent (Thermofisher, USA) using a protocol adapted from *Piper et al.* (2008) using the miRNeasy mini kit (QIAGEN, USA) as per manufacturer's instructions. RNA samples were treated with

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DNAse and RNA was quantified using the Nanodrop 2000 (Thermofisher, USA). The RNA RIN quality analysis was evaluated with the 2100 Bioanalyzer Instrument (Agilent Technologies, USA). The cDNA libraries were prepared with the TruSeq Stranded Total RNA kit with Ribo-depletion (Illumina, USA) and sequenced as 100 bp paired-end reads on one full S1 flowcell on the NovaSeq 6000 sequencer (Illumina, USA) through the Australian Genome Research Facility (Melbourne, AUS).

Bioinformatic pipeline. Quality control, mapping and quantification of RNA reads was performed with methods described previously (Mantilla Valdivieso *et al.* 2021) using the ARS_UCD1.2 genome and HISAT2 as the mapper tool. Gene expression was modelled with the edgeR package in RStudio (Robinson, McCarthy and Smyth 2009) using the trimmed mean of M-values (TMM) method. The linear model ~MTS+TPS+BIC+RIN was implemented to test for differentially expressed genes in the comparison of animals of low versus high mean tick score (MTS) accounting for the effects of timepoint tick score on week 12 (TPS), the proportion of individual *Bos indicus* content (BIC) (Hayes, Fordyce and Landmark 2019), and the RIN quality of the sample (RIN). Differentially expressed genes (DEGs) were considered significant based on a false discovery rate (FDR)<0.05. RIF analysis (Reverter *et al.* 2010) was implemented through the CeTF package in RStudio (Oliveira de Biagi *et al.* 2020) using filtered and normalised gene expression matrices (logCPMs) as input. The list of TFs and cofactors were obtained from the AnimalTFDBB bovine database (http://bioinfo.life.hust.edu.cn/AnimalTFDB/#!/download) to identify expressed TFs in our data. Functional enrichment of KEGG pathways was performed with the ClusterProfiler R package (Wu *et al.* 2021).

Results

A total of 17512 genes were expressed in cattle skin of low versus high host resistance Brangus cattle. EdgeR identified 229 significantly differentially expressed genes (DEGs; FDR<0.05) of which 128 were upregulated and 101 downregulated. Of these, 212 were target genes for 1866 transcription factors (TFs) expressed in skin (Figure 1A). Regulatory Impact Factor (RIF) analysis to predict key regulatory elements showed 158 significant TFs (*P*-value<0.05) based on metrics RIF1 and RIF2 metrics. Briefly, RIF1 captures TFs showing differential connectivity to DEGs found between high and low resistance phenotypes, whereas RIF2 focuses on TFs showing evidence as predictors of change in abundance of these DEGs. Furthermore, 2 transcription

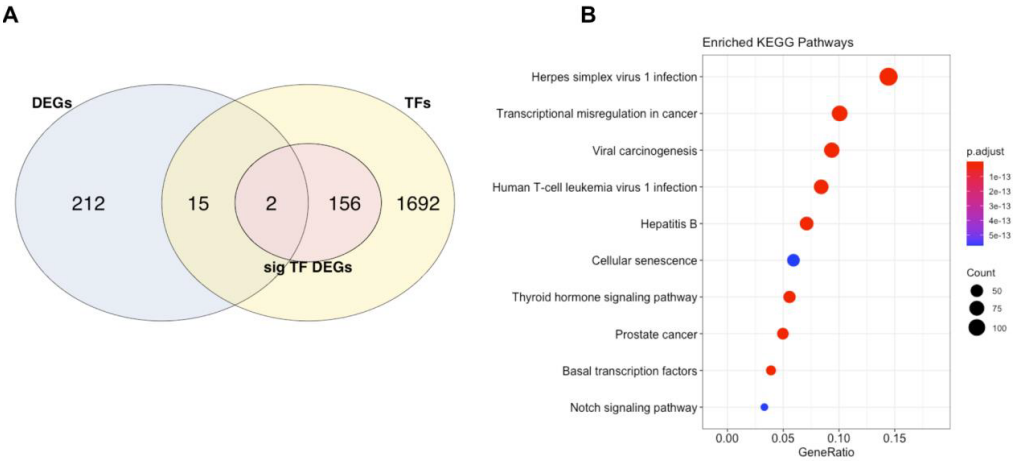

Figure 1. (A) Unique and common genes between sets of skin DEGs, expressed TFs, and significant TFs (by RIF and DEG). (B) Enriched KEGG biological pathways in the set of skin DEGs and significant TFs.

Table 1. Transcription factors identified as significant by regulatory impact factor metrics (RIF1 and RIF2, $P<0.05$) and differential gene expression (FDR<0.05).

TF symbol	Name	RIF1[1]	RIF2[1]	logFC[2]	FDR[3]
GRHL3	grainyhead like transcription factor 3	0.50	-2.68	1.24	0.048
DTX1	deltex E3 ubiquitin ligase 1	-0.48	-1.96	-2.26	0.017

[1] RIF significance value based on z-scores above 1.96 standard deviation from the mean.
[2] FC = log2 fold change between low and high tick resistance animals.
[3] FDR = false discovery rate corrected (Benjamini-Hochberg) *P*-values.

factor genes *GRHL3*, and *DTX1* were found to be significant by both RIF metrics and differential genes expression in this experiment (Table 1). Functional biological enrichment of the significant TFs and DEGs (2077 genes) showed that the most represented pathways were related to immune response to infection and transcriptional misregulation in cancer (Figure 1B). Enriched pathways with fewer genes included basal transcription factors and the Notch signalling pathway.

Discussion

Artificial challenge with *R. australis* ticks promoted divergent phenotypes of host resistance as determined by their average tick burden during the infestation experiment. Skin transcriptomic data revealed 229 significant DEGs and 158 significant TFs in the comparison of low versus high host resistance Brangus steers. Of these, *GRHL3* (grainy head like transcription factor 3) and *DTX1* (deltex E3 ubiquitin ligase 1) were identified as key regulators by both differential expression and RIF analysis, with high RIF2 metric indicating these genes are potential useful predictors of change in abundance of the skin DEGs. *GRHL3* is an epidermal transcription factor which regulates the differentiation and migration transitions of the progenitor epidermal cell (Klein *et al.* 2017). The regulation of these steps in the epidermal differential process appears to be relevant during wound healing and tissue homeostasis. The upregulation of *GRHL3* in the skin of low host resistance cattle suggests participation in the modulation of gene expression in response to tick infestation. On the other hand, *DTX1* is a transcriptional factor target of the Notch signalling cascade with possible immune effects (Brandstadter and Maillard 2019). Likewise, functional enrichment analysis captured both immune and transcriptional regulation pathways overrepresented in the significant DEGs and TFs including the Notch signalling pathway, which plays multiple functions in the innate and adaptive immune cell development and is consequently relevant in host defence. Overall, these results contribute towards uncovering some of the biology behind this complex trait putting forward a list of candidate regulator genes that may be drivers of differential expression of tick resistance in cattle. Further cross-validation of the significant DEGs and TFs with other skin or blood gene expression studies will facilitate the identification of biomarkers of tick resistance. In this way, improved phenotyping approaches with predictive markers will provide an opportunity rapidly build reference populations for genetic evaluation in breeding programs for tick-endemic regions.

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