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An In silico study on protein-protein interactions in dairy cows with mastitis

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Abstract

Mastitis is one of the important factors of profit reduction in dairy farms. The study of gene expression in infected cows can identify candidate genes for use in genomic selection programs or even the invention of effective medicines. RNA-seq studies determine the expression of numerous genes simultaneously. In this study, the output of several researches related to gene expression analysis with RNA-seq from mammary tissue of dairy cows with mastitis were investigated, and protein-protein interaction (PPI) network was constructed by STRING and analyzed by Network Analyzer. Based on degree of each protein, it was showed that IL18, LDH, IL10, B2M, CD74 and BoLA-DRA proteins had the highest PPI score and thus could play a key role in mastitis in dairy cows.

Keywords: mastitis, dairy cow, RNA-seq, protein, STRING

Introduction

Mastitis is one of the main reasons of profit loss in dairy farms. The cause of the disease is the penetration of bacteria into the udder and stimulation of the immune system, and inflammation of udder tissues (Cheng et al., 2021). Mastitis occurs in two types: clinical with specific symptoms and sub-clinical without specific symptoms. For treatment, the implementation of antibiotics is effective, but due to the limitation of the use of antibiotics in animal feed as well as bacterial resistance, it is necessary to find more suitable ways for prevention of the disease. One of the effective ways to prevent the disease is to improve the animal's natural defense mechanism (Asselstine et al., 2019). There are several reports

regarding the investigation of gene transcription using RNA-seq in dairy cows. The aim of this study was to analyze the interaction of proteins identified with high expression in cows with mastitis from RNA-seq studies, using the STRING server.

Materials and Methods

In this study, articles related to gene expression in cows with mastitis were collected, and genes that were differentially expressed larger than two fold in infected cows compared to healthy individuals were selected (Asselstine et al., 2019; Cheng et al., 2021; Fang et al., 2017; Farmanullah et al., 2021; Kaisa et al., 2021; Naserkheil et al., 2022; Passe Pereira et al., 2021). The protein names were input into the STRING server version 11.5 (https://string-db.org). Then the network was analyzed by Network analyzer in order to rank the genes (Saedi et al., 2022). Functional enrichments including gene ontology, protein-protein interaction (PPI) and KEGG pathways which were evaluated considering no change in defaults of the server except for clustering in which three clusters were selected.

Results and Discussion

In total, 243 up-regulated genes in cows with mastitis were selected which showed above two fold change. The network analysis of PPI showed many interactions between proteins. 237 nodes with 1461 edges were determined. The average degree of each node was 12.3. The result of functional enrichment analysis is shown in Table 1. The three main GO categories were analyzed using the list of 243 DE genes. A total of 664, 43, and 23 significantly enriched GO terms were identified in the biological process (BP), molecular function, and cellular component, respectively. Six BPs had *strength* equal to 1.93 including interleukin-18-mediated signaling pathway, Positive regulation of macrophage migration inhibitory factor signaling pathway, Regulation of leukocyte adhesion to arterial endothelial cell, Cellular response to triacyl bacterial lipopeptide, Neutrophil aggregation, T-helper 1 cell cytokine production.
Three proteins were involved in the interleukin-18-mediated signaling pathway (*FDR*< 0.001). Three proteins included in Lipopolysaccharide immune receptor activity (*FDR*< 0.008) and four proteins in the Anchored component of external side of plasma membrane with *FDR*< 0.008 and *strength* equal to 1.35, which describes how large the enrichment effect is. IL18RAP, NCF2 and HCK proteins are active in the biological process of interleukin 18 (red nodes). CXCR4 and CD74 proteins (yellow nodes) are involved in macrophage activity. IL-18 RAP is a component of the IL-18 pro-inflammatory cytokine receptor. NCF2 is a subunit of the NADPH oxidase enzyme complex, which is involved in phagocytic activity and regulation of neutrophil activity (https://medlineplus.gov). HCK protein plays an important role in innate immunity such as regulating the activity of neutrophils, monocytes, macrophages, phagocytosis and cell migration (https://www.uniprot.org).

Regarding molecular functions, the main GO terms were catalytic activity and binding activity. This was in line with the results by Asselstine et al. (2019). IL8, IL10, IL18, and CTSC were reported as biomarkers for *E. coli* mastitis and were related to the immune response of bovine mastitis. CTSC plays a role in the producing of cytotoxic lymphocyte (Farmanullah et al. 2021). Previous studies revealed that blocking of cell surface CXCR1 expression might be used as an effective treatment against *S. aureus* infection (Dutta and Bishay, 2020; Wang et al. 2020). When the process of inflammation and cell damage in udder occurs, LDH is released from the cell into the milk. Therefore, *LDH* expression increases in infected cows (Jorgensen et al. 2016). Furthermore, the three genes of *B2M*, *CD74* and *BoLA-DRA* are directly related to major histocompatibility complex, which is an essential part of the immune system and accelerates the identification of antigens and the immune response (Behl et al. 2012). As shown in Figure 1, IL10 is at the center of the PPI network and interacts with members of immune system proteins. IL10 can improve inflammation or inhibit it.

LDH protein can be introduced as a mastitis biomarker. Also, *IL18*, *B2M*, *CD74*, *BoLA-DRA* and *IL10* can be candidate genes in resistance to mastitis. However, more study is needed to identify SNPs and their specific roles in the disease.

Table 1. Associated Gene Ontology (GO) terms with differentially expressed genes in healthy and mastitic samples (*FDR*< 0.05, fold change > 2) in the GO categories (a) biological process, (b) molecular function, and (c) cellular component

Description	Count in network	*strength*	*FDR*
(a) Biological Process			
interleukin-18-mediated signaling pathway	3 of 3	1.93	0.00096
Positive regulation of macrophage migration inhibitory factor signaling pathway	2 of 2	1.93	0.0162
Regulation of leukocyte adhesion to arterial endothelial cell	2 of 2	1.93	0.0162
Cellular response to triacyl bacterial lipopeptide	2 of 2	1.93	0.0162
Neutrophil aggregation	2 of 2	1.93	0.0162
(b) Molecular Function			
Lipopolysaccharide immune receptor activity	3 of 4	1.8	0.0083
Ammonia-lyase activity	3 of 5	1.7	0.0116
Glucose binding	3 of 7	1.56	0.0185
Activin-activated receptor activity	3 of 8	1.5	0.0223
Macrolide binding	3 of 8	1.5	0.0223
(c) Cellular Component			
Transcription factor ap-1 complex	2 of 2	1.93	0.0460
Activin receptor complex	3 of 6	1.63	0.0106
Anchored component of external side of plasma membrane	4 of 15	1.35	0.0076

Anchored component of plasma membrane	5 of 44	0.98	0.0212
External side of plasma membrane	17 of 286	0.7	266e-05

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