6-11-2019

Prediction of key regulators and downstream targets of E. coli induced mastitis

Somayeh Sharifi
Iowa State University

Abbas Pakdel
Isfahan University of Technology

Esmaeil Ebrahimie
The University of Adelaide

Yalda Aryan
Payam Noor University

Mostafa Ghaderi Zefrehee
Yasouj University

See next page for additional authors

Prediction of key regulators and downstream targets of E. coli induced mastitis

Abstract
Mastitis, an inflammatory response of mammary glands to invading bacteria, is one of the most economically costly diseases affecting dairy animals. E. coli can introduced as a major etiological agent of bovine mastitis in wellmanaged dairy farms. It is of great significance to understand the regulatory mechanisms by which the disease can be controlled. High-throughput technologies combined with novel computational systems biology tools have provided new opportunities for better understanding the molecular mechanisms that underlie disease. In the current study, the results of microarray meta-analysis research were used to perform a network analysis to potentially identify molecular mechanisms that regulate gene expression profile in response to E. coli mastitis. In our result, transcription factors, TP53, SP1, ligands, INS, IFNG, EGF, and protein kinases, MAPK1, MAPK14, AKT1, were identified as the key upstream regulators whereas protein kinases, MAPK3, MAPK8, MAPK14, ligands, VEGFA, IL10, an extracellular protein, MMP2, and a mitochondrial membrane protein, BCL2, were identified as the key downstream targets of differentially expressed genes. The results of this research revealed important genes that have the key functions in immune response, inflammation or mastitis which can provide the basis for strategies to improve the diagnosis and treatment of mastitis in dairy cows.

Keywords
Dairy cattle, Escherichia coli, Functional genomics, Gene network, System biology, Transcriptome

Comments
This is a manuscript of an aritcle published as Sharifi, Somayeh, Abbas Pakdel, Esmaeil Ebrahimie, Yalda Aryan, Mostafa Ghaderi Zefrehee, and James M. Reecy. "Prediction of key regulators and downstream targets of E. coli induced mastitis." *Journal of applied genetics* (2019). doi: 10.1007/s13353-019-00499-7. Posted with permission.

Authors
Somayeh Sharifi, Abbas Pakdel, Esmaeil Ebrahimie, Yalda Aryan, Mostafa Ghaderi Zefrehee, and James M. Reecy

Prediction of key regulators and downstream targets based on global differentially expressed genes in *E. coli* induced mastitis

Somayeh Sharifi[1,2], Abbas Pakdel[1], Esmaeil Ebrahimie[3,4,5,6], Yalda Aryan[7], Mostafa Ghaderi Zefrehee[8], James M. Reecy[2*]

1. *Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.*
2. *Department of Animal Science, Iowa State University, Ames, Iowa, United States of America.*
3. *Adelaide Medical School, The University of Adelaide, Adelaide, Australia.*
4. *Australian Centre for Antimicrobial Resistance Ecology, School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA, Australia.*
5. *Division of Information Technology, Engineering and the Environment, School of Information Technology and Mathematical Sciences, University of South Australia. Adelaide, South Australia, Australia.*
6. *School of Biological Sciences, Faculty of Science and Engineering, Flinders University, Adelaide, South Australia, Australia.*
7. *Computer Engineering Payam Noor University, Tehran, IRAN.*
8. *Department of Animal Science, College of Agriculture, Yasouj University, Kohgiluyeh* and Boyer-Ahmad, Iran.

Corresponding author: jreecy@iastate.edu

Department of Animal Science 1221 Kildee Hall

Iowa State University Ames, Iowa 50011-3150

phone: (515) 294-9262

fax: (515) 294-6994

Abstract

Mastitis, an inflammatory response of mammary glands to invading bacteria, is one of the most economically costly diseases affecting dairy animals. *E. coli* can introduced as a major etiological agent of bovine mastitis in well-managed dairy farms. It is of great significance to understand the regulatory mechanisms by which the disease can be controlled. High-throughput technologies combined with novel computational systems biology tools have provided new opportunities for better understanding the molecular mechanisms that underlie disease. In the current study, the results of microarray meta-analysis research were used to perform a network analysis to potentially identify molecular mechanisms that regulate gene expression profile in response to *E. coli* mastitis. In our result, transcription factors, *TP53*, *SP1*, ligands, *INS, IFNG, EGF,* and protein kinases, *MAPK1, MAPK14*, *AKT1,* were identified as the key upstream regulators whereas protein kinases, *MAPK3, MAPK8*, *MAPK14*, ligands, *VEGFA*, *IL10,* an extracellular protein, *MMP2*, and a mitochondrial membrane protein, *BCL2,* were identified as the key downstream targets of differentially expressed genes. The results of this research revealed important genes that have the key functions in immune response, inflammation or mastitis which can provide the basis for strategies to improve the diagnosis and treatment of mastitis in dairy cows.

Keywords: Dairy cattle, Escherichia coli, Functional genomics, Gene network, System biology, Transcriptome.

1. INTRODUCTION

It is of great significance to understand the regulatory mechanisms by which the disease can be controlled. Novel computational systems biology tools such as the discovery of pathways based on enrichment analysis, upstream regulator or downstream targets discovery, meta-analysis, machine learning and pattern recognition, have provided new opportunities to understand the molecular mechanisms of diseases and system biology (Alanazi and Ebrahimie 2016; Kargarfard et al. 2015; Sharifi et al. 2018; Subramanian et al. 2005). This knowledge could lead to improve the diagnosis and treatment of different diseases and provide the mechanistic insights into host resistance in an efficient way.

Mastitis, the inflammatory response of mammary glands to invading bacteria, is one of the most economically important diseases affecting dairy animals (Bar et al. 2008; Hogeveen et al. 2011). Mastitis control programs based on milking time hygiene, antibiotic therapy, and culling of persistently infected cows has impacted the prevalence of contagious mastitis pathogens, such as *Staphylococcus aureus* and *Streptococcus agalactiae* in various regions. However, these procedures have had a marginal effect on environmental pathogens such as *E. coli*. Thus, environmental mastitis pathogens have become a major etiological agent of bovine mastitis in well-managed dairy farms (Bradley 2002; Hogan and Larry Smith 2003). *E. coli* infection causes acute inflammation with clinical signs in dairy cows, which however may be self-healing by eventually eradicating the invader, are occasionally fatal (Bannerman et al. 2004; Burvenich et al. 2007; Hagiwara et al. 2016). More recently, numerous studies have analyzed the transcriptome profile of the bovine mammary gland in response to infection (Rinaldi et al. 2010; Sipka et al. 2014; Younis et al. 2016). Such tests allow for the identification of biomarkers, disease-causing genes, gene expression profiles, and provide an understanding of complex molecular mechanisms in cell physiology and pathology, which help to improve diagnosis, prognosis, and monitoring of responses to therapy. Meta-analysis is a relatively inexpensive option that has the potential to increase the statistical power and generalizability of single-

study analysis. Furthermore, it can bypass challenges associated with individual variations, such as a small number of samples and strengthen the mildest data perturbations (Mimoso et al. 2014; Ramasamy et al. 2008; Sharifi et al. 2018). In the previous meta-analysis studies, a common transcriptional response in the mammary glands of several species to different pathogens was identified. Moreover, several new pathways were distinguished, which not previously identified in the individual studies (Genini et al. 2011) and comprehensively investigated differences in transcriptional responses between different agents of induced mastitis (Younis et al. 2016). In our previous meta-analysis study of mastitis gene expression data, two data mining tools, meta-analysis, and machine learning were integrated to provide an opportunity to identify important genes that had a robust bio-signature and thereby may be good biomarker candidates in response to *E. coli* mastitis (Sharifi et al. 2018). Here, to have a better understanding of the molecular mechanisms that underlie the results achieved from our previous research, we designed a study to identify the key regulators and targets of transcriptomic response in *E. coli* mastitis. In the systems biology, the common regulators and down-stream target discovery based on enrichment analysis approach are reliable approaches to obtain a comprehensive view of the molecular mechanisms, so that a specific transcriptome and proteome profile can promote (Alanazi and Ebrahimie 2016; Bakhtiarizadeh et al. 2013). This study has provided a comprehensive insight into mastitis disease based on computational systems biology.

2. MATERIALS AND METHOD

2.1. Differentially expressed genes achieved from the meta-analysis

The expression profile of the bovine mammary gland in response to *E. coli* infection from 6 independent microarray-based studies have been previously studied (Sharifi et al. 2018). In brief, 130 mammary gland samples (57 healthy and 73 infected) from 15 datasets in 6 studies were included in the differential gene expression analysis. After quality control and quartile normalization and summarization of each dataset by RMA, the gene expression levels from mastitis and healthy samples in each dataset were compared using a moderated Student's t-test. The *p-values* of each dataset were combined with the rth ordered *p-value* (rOP) meta-analysis method. The eight hundred and eighty-five genes were identified as differentially expressed genes introduced by meta-analysis (meta-genes), from which 16 % (143 genes) were down-regulated and 84 % (742 genes) were up-regulated (one-tailed, $q<0.005$) (additional data are given in Online Resource 1).

2.2. Common Targets and Common Regulators Algorithms

Meta-genes were used as the input of Common Targets and Common Regulators algorithms. The Common Regulators algorithm were used to identify the gene regulators that have the highest number of regulation/expression relationships/ miRNA effect and promoter binding with meta-genes. In contrast, the Common Targets algorithm was used to identify the targets and molecular mechanisms that are activated/inactivated by the altered expression of meta-genes (Alanazi and Ebrahimie 2016; Gholizadeh-Ghaleh Aziz et al. 2016; Panahi et al. 2015). Pathway Studio web tool 12.0.1.5 was used to identify the key targets and the key regulators, as previously described (Alanazi and Ebrahimie 2016; Gholizadeh-Ghaleh Aziz et al. 2016; Panahi et al. 2015). Pathway Studio is a pathway analysis tool that utilizes the ResNet Mammal database, which incorporates a number of public and commercial databases such as KEGG (Kanehisa 2008), BIND (Bader et al. 2003), and GO (Ashburner et al. 2000) and to obtain the latest

information from PubMed and other public sources use the powerful text-mining tool MedScan (Elsevier-Ariadne Genomics, Rockville, MD) (Nikitin et al. 2003). Gene networks were constructed using the proteins and complexes as an entity, while gene expression, miRNA effect, promoter binding, and regulation were selected as relation types. For a component to be identified as a key regulator or key target, it had to have a high number of upstream or downstream interactions with meta-genes respectively. After testing different values, a threshold of 70 interactions for Common Regulators and 60 interaction for Common Targets algorithms were selected. For more confidence, all relationships that were reported in more than 5 references were selected. To find more effective and specific key regulators or targets, components which commonly present in many biological processes or networks were removed from the analysis. For this purpose, 20 randomized gene collections with 885 genes (number of meta-genes) from the Affymetrix bovine GeneChipTM platform ("http://www.affymetrix.com/index.affx" Accessed February 2016) were selected and the whole process in drawing networks was performed on them. Then the genes which were identified as key regulators or key targets in these random sets were removed from the original networks. In the last network, effective and specific key regulators and targets that had a roll in mastitis were more clearly identified.

3. RESULTS

3.1. Key regulators of differentially expressed meta-genes in E. coli mastitis

The network constructed by the Common Regulators algorithm based on the 885 meta-genes after removal of components that were identified by analysis of random gene collections is presented in Fig. 1.

Fig. 1 Regulator discovery of differentially expressed genes achieved from meta-analysis in the course of *E. coli* mastitis based on common regulator algorithm with interaction threshold of 70. Up-regulated genes are presented as red highlighted nodes and down-regulated gene are denoted as green nodes

Eight genes that were determined as the key upstream regulators, with a high number of regulatory interactions (with at least 70 downstream interactions) on the meta-genes, are listed in Table 1. All of the relationships and underlying references of these networks are presented in Online Resource 2.

Table 1 Key gene regulators for meta-genes in *E. coli* mastitis based on Common Regulators algorithm with the interaction threshold of 70

Gene symbol	Full gene name	Functional group	Cellular component
MAPK1	Mitogen-activated protein kinase	Protein kinase	Cytoplasm, cytoskeleton, spindle
TP53 (p53)	Tumor protein p53	Transcription factor	Cytoplasm
SP1	Sp1 transcription factor	Transcription factor	Nucleus
MAPK14	Mitogen-activated protein kinase 14	Protein kinase	Cytoplasm
INS	Insulin	Ligand	Secreted
EGF	Epidermal growth factor	Ligand	Membrane
AKT1	AKT serine/threonine kinase 1	Protein kinase	Cytoplasm
IFNG	Interferon gamma	Ligand	Secreted

3.2. Key down-stream targets of meta-genes in E. coli mastitis

The network constructed by the Common Targets algorithm is presented in Fig. 2.

Fig. 2 Targets of differentially expressed genes achieved from meta-analysis in the course of *E. coli* mastitis based on common target algorithm with interaction threshold of 60. Up-regulated genes are presented as red highlighted nodes and down-regulated gene are denoted as green nodes

All the relationships and underlying references of these networks are presented in additional data are given in Online Resource 3. Seven genes that showed a high number of interactions (with at least 60 downstream interactions) regulated by meta-genes were identified as the key downstream targets and are listed in Table 2.

Table 2 Key targets genes of meta-genes in *E. coli* mastitis based on Common Target algorithm with the interaction threshold of 60

Gene symbol	Full gene name	Functional group	Cellular component
MAPK3	Mitogen-activated protein kinase 3	Protein kinase	Cytoplasm
MAPK8	Mitogen-activated protein kinase 8	Protein kinase	Cytoplasm
VEGFA	Vascular endothelial growth factor A	Ligand	Secreted
MMP2	Matrix metallopeptidase 2		Secreted, extracellular space, extracellular matrix
BCL2	BCL2, apoptosis regulator		Mitochondrion outer membrane
MAPK14	Mitogen-activated protein kinase 14	Protein kinase	Cytoplasm
IL10	Interleukin 10	Ligand	Secreted

4. **DISCUSSIONS**

Eight key common regulators that identified based on network analysis are listed in Table 1. Transcription factors including *TP53* and *SP1*, ligands such as *INS, IFNG, EGF* and protein kinases *MAPK1, MAPK14* and *AKT1* were identified as key regulators as they exhibited most regulatory relations with meta-genes (see S2 Table for more details about all relations and references). The first regulator, *TP53* (*P53*) transcription factor, acts through suppression of *NF-κB* and thereby serving as a negative regulator of inflammation by "buffering" innate immune responses (Cooks et al. 2013; Gudkov et al. 2011). It has been investigated that the LPS challenge induced changes in liver lipid composition, systemic inflammation (rise of blood ceruloplasmin and bilirubin), and activation of the *p53* signaling pathway and suggested that the up-regulation of this transcription factor is a quick response by the liver to maintain homeostasis (Minuti et al. 2015).

SP1, another identified key regulator, is the founding member of the *Sp* transcription factor family. Members of this family contain C2H2-type zinc fingers (Black et al. 2001). Investigations have revealed that this transcription factor both activates and suppresses the expression of a number of essential oncogenes and tumor suppressors, as well as genes involved in essential cellular functions, including proliferation, differentiation, the DNA damage response and apoptosis (Beishline and Azizkhan-Clifford 2015). Previous studies that have investigated the decreased inflammatory signaling observed in response to non-steroidal anti-inflammatory drugs (NSAID) treatment have shown that this decreased response is associated with decreased Sp1 protein levels, as well as in Sp1-dependent transcriptional activity (Abdelrahim and Safe 2005; Pan and Hung 2002; Pathi et al. 2012; Pathi et al. 2014; Wei et al. 2004). Furthermore, it has been shown that gene therapy that targets the suppression of *NF-κB* and *Sp1* might provide a new target for the prevention of inflammatory disease (Lee et al. 2013).

Insulin, another identified regulator, has been shown to reduce considerable several key mediators of oxidative, nitrosative and inflammatory stress and reduce tissue damage induced by LPS (Dandona et al. 2010). Furthermore, it has been reported that Insulin suppresses the expression of several TLRs, which are major determinants of the inflammatory response to viral and bacterial pathogens, at the transcriptional level. In addition, low-dose insulin infusion has been shown to exert a prompt and powerful anti-inflammatory effect (Dandona et al. 2001; Ghanim et al. 2008). Insulin has been investigated for its potential use as an anti-inflammatory therapy for endotoxemia (Chaudhuri and Umpierrez 2012; Dandona et al. 2010; Ghanim et al. 2008).

Functional *EGF* receptors (EGFR, ErbB1/HER-1) on peripheral blood monocytes and monocyte-derived macrophages mediate both chemotaxes in monocytes and macrophages and proliferation of macrophages (Lamb et al. 2004; Shafi et al. 2010). Moreover, in some studies, *EGF* has been identified as the specific biomarker for inflammation (Centola et al. 2013; Rech et al. 2016).

The known pro-inflammatory cytokine, interferon-gamma (IFNG) play a complex modulatory role in immune defense during microbial infections (Too et al. 2014; Turner et al. 2014).

The last regulators, *MAPK1* and *MAPK14,* which are members of MAPKs protein kinases, play a central role in inflammatory responses and autoimmune diseases. Previous studies have demonstrated that *NF-kB* and *MAPK*s were activated in LPS-induced mouse mastitis, and more importantly, the inhibitory action of some treatments on the activation of NF-kB and MAPKs blocked the up-regulation of pro-inflammatory cytokines (He et al. 2015; Li et al.

2013). Another protein kinase, *AKT1,* which was identified as the key regulator of mastitis in this study, is a member of the AKT serine-threonine protein kinase (also known as PKB) family. AKTs family members are activated by PI-3K and play the key regulatory roles in a host of cellular functions including cell survival, cell proliferation, differentiation, and intermediary metabolism (Androulidaki et al. 2009). Previous investigations have revealed that *Akt1* suppresses acute inflammatory responses and that inhibition of AKT1 may modulate innate immunity and inflammation (Androulidaki et al. 2009; Arranz et al. 2012; Di Lorenzo et al. 2009). In addition, *Akt1* has been identified as a key molecule for the regulation of LPS responsiveness and tolerance. AKT1 can regulate miRNAs that control TLR4 and fine-tune SOCS1 expression. As a result, compounds that can systemically inhibit *Akt1* may be able to be developed as drugs (Androulidaki et al. 2009).

Seven key targets genes of meta-genes in *E. coli* mastitis based on Common Targets algorithm listed in Table 2 including protein kinase, *MAPK3, MAPK8* and *MAPK14*, extracellular protein, *MMP2*, ligands such as *VEGFA* and *IL10* and also mitochondrion membrane protein, *BCL2* (refer S3 Table for more details about all relations and references). *MAP kinases* have identified as both key regulator and target in our study and their role in inflammatory responses and autoimmune diseases were previously discussed. MAP kinases are one of most ancient signal transduction pathways and are widely used throughout evolution in many physiological processes. In mammalian species, MAP kinases play important roles in all aspects of immune responses: from the initiation phase of innate immunity, to activation of adaptive immunity, and to cell death when immune function is complete. It has been shown that MAP kinases are subject to the context of different cell types and different immune responses by collaborating with each other or with other signal transduction pathways (Dong et al. 2002).

Matrix metalloproteinases (MMPs) are a group of zinc-dependent enzymes that regulate extracellular matrix (ECM) turnover during both normal physiological processes and under pathological conditions, such as inflammation (Papageorgiou and Heymans 2012). Matrix metalloproteinases are able to influence inflammation at multiple levels. They can cleave adhesion molecules, which facilitates cell migration through the endothelial cell junction (Endo et al. 2003; McGuire et al. 2003). In addition to their ability to facilitate leukocyte migration through the ECM, MMPs, produced either by neutrophils (e.g. MMP-2, MMP-3, MMP-9) or macrophages (MMP-2, MMP-9, MMP-12), can modulate inflammation by affecting the bioavailability of cytokines and chemokines (Soehnlein et al. 2010). Previous studies have revealed that MMP inhibitors can be used as anti-inflammatory drugs (Lee et al. 2004; Manicone and McGuire 2008; Papageorgiou and Heymans 2012; Parks et al. 2004). Lymphangiogenesis is an important physiological response to inflammatory stimuli. It acts to limit inflammation (Tan et al. 2013). Inflammatory lymphangiogenesis is principally driven by vascular endothelial growth factor (VEGF)-A via contribution by neutrophils (Tan et al. 2013) and macrophage recruitment (vegfa_2). Understanding how inflammatory lymphangiogenesis is regulated has been shown to be essential to develop innovative therapies for inflammatory disorders (Tan et al. 2013).

Interleukin 10 (*IL-10*), a well-recognized anti-inflammatory cytokine, is produced by lymphocytes, B cells, and monocytes (Mizia-Stec et al. 2003). *IL-10* has been identified as the appropriate biomarker for inflammation disease (Malarstig et al. 2008; Zhang et al. 2015).

Another key target identified in our study was *BCL2.* It is an anti-apoptotic protein, which suppresses activation of *caspase-1,* which reduces production of the *caspase-1* substrate interleukin-1b (Bruey et al. 2007; Kobayashi et al. 2007; Portt et al. 2011). Modulation of the apoptotic machinery, a form of programmed cell death, during viral and bacterial infections, is a well-established mechanism that promotes the survival of affected cells (Busca et al. 2009b; Song et al. 2014). Anti-apoptotic genes such as *Bcl2* family members have been shown to be involved in the survival of monocytes/macrophages through enhancing the resistance of macrophages against various apoptotic stimuli. Because of the crucial role of macrophages in immunity and innate immunity, regulation of monocyte/macrophage lifespan is important in both physiological and pathological processes. Understanding how anti-apoptotic agents contribute to the enhanced survival of infected macrophages provides a basis for therapeutic strategies (Busca et al. 2009a).

As discussed previously all of the key regulators or key targets introduced by this research have important roles in inflammation, immune system or mastitis. The identification of disease-causing genes and their use as biomarkers has been shown to improve diagnosis, prognosis, and monitoring of responses to therapy (Lewis 2011; Mohammadi et al. 2011).

CONCLUSION

This study utilized systems biology tools to identify the key regulators and targets by investigating Common Targets and Common Regulators algorithms while using the power of meta-analysis to detect differentially expressed genes in *E. coli* mastitis. Complementary information from the reliable source of information such as gene network help us to get the better underestanding of functional genomics of genes. Interestingly, all of the key regulators or key targets introduced by this research had been shown important roles in inflammation, immune system or mastitis. Identification of key genes that underlie complex traits such as susceptibility to mastitis is an opportunity to improve the diagnosis and treatment of diseases and provide mechanistic insights into host resistance in an efficient way.

SUPPLEMENTARY MATERIAL

Online Resource 1. Differentially expressed genes identified after meta-analysis (one-tailed $q<0.005$).

Online Resource 2. The details of applying of Common Regulator algorithms on meta-genes (interaction threshold of 70 which reported by more than 5 references)

Online Resource 3. The details of applying of Common Target algorithms on meta-genes (interaction threshold of 60 which reported by more than 5 references)

COMPLIANCE WITH ETHICAL STANDARDS:

Research is not involving Human Participants and/or Animals.

FUNDINGS:

The authors received no specific funding for this work.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exist.

REFERENCES

Abdelrahim M, Safe S (2005) Cyclooxygenase-2 inhibitors decrease vascular endothelial growth factor expression in colon cancer cells by enhanced degradation of Sp1 and Sp4 proteins Mol Pharmacol 68:317-329 doi:10.1124/mol.105.011825

Alanazi IO, Ebrahimie E (2016) Computational Systems Biology Approach Predicts Regulators and Targets of microRNAs and Their Genomic Hotspots in Apoptosis Process Mol Biotechnol 58:460-479 doi:10.1007/s12033-016-9938-x

Androulidaki A et al. (2009) The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs Immunity 31:220-231 doi:10.1016/j.immuni.2009.06.024

Arranz A et al. (2012) Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization Proc Natl Acad Sci U S A 109:9517 9522-doi:10.1073/pnas.1119038109

Ashburner M et al. (2000) Gene ontology. Tool for the unification of biology. The Gene Ontology Consortium. doi:http://www.geneontology.org

Bader GD, Christopher DB, Hogue WV (2003) BIND. The biomolecular interaction network database. doi:http://bind.ca

Bakhtiarizadeh MR, Moradi-Shahrbabak M, Ebrahimie E (2013) Underlying functional genomics of fat deposition in adipose tissue Gene 521:122-128 doi:10.1016/j.gene.2013.03.045

Bannerman DD, J. PM, Jai-Wei L, Xin Z, Hope JC ,P. R (2004) Escherichia coli and Staphylococcus aureus Elicit Differential Innate Immune Responses following Intramammary Infection Clin Diagn Lab Immunol 11(3):463–472

Bar D et al. (2008) The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming J Dairy Sci 91:2205-2214 doi:10.3168/jds.2007-0573

Beishline K, Azizkhan-Clifford J (2015) Sp1 and the 'hallmarks of cancer' FEBS J 282:224-258 doi:10.1111/febs.13148

Black AR, Black JD, Azizkhan-Clifford J (2001) Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer J Cell Physiol 188:143-160 doi:10.1002/jcp.1111

Bradley A (2002) Bovine mastitis: an evolving disease Vet J 164:116-128

Bruey JM et al. (2007) Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1 Cell 129:45-56 doi:10.1016/j.cell.2007.01.045

Burvenich C, Bannerman DD, Lippolis JD, Peelman L, Nonnecke BJ, Kehrli ME, Jr., Paape MJ (2007) Cumulative physiological events influence the inflammatory response of the bovine udder to Escherichia coli infections during the transition period J Dairy Sci 90 Suppl 1:E39-54 doi:10.3168/jds.2006-696

Busca A, Saxena M, Kryworuchko M, Kumar A (2009a) Anti-Apoptotic Genes in the Survival of Monocytic Cells During Infection Curr Genomics 10:306-317

Busca A, Saxena M, Kryworuchko M, Kumar A (2009b) Anti-apoptotic genes in the survival of monocytic cells during infection Curr Genomics 10:306-317 doi:10.2174/138920209788920967

Centola M et al. (2013) Development of a multi-biomarker disease activity test for rheumatoid arthritis PLoS One 8:e60635 doi:10.1371/journal.pone.0060635

Chaudhuri A, Umpierrez GE (2012) Oxidative stress and inflammation in hyperglycemic crises and resolution with insulin: implications for the acute and chronic complications of hyperglycemia J Diabetes Complications 26:257-258 doi:10.1016/j.jdiacomp.2012.04.016

Cooks T et al. (2013) Mutant p53 prolongs NF-kappaB activation and promotes chronic inflammation and inflammation-associated colorectal cancer Cancer Cell 23:634-646 doi:10.1016/j.ccr.2013.03.022

Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S (2001) Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 86:3257-3265 doi:10.1210/jcem.86.7.7623

Dandona P, Ghanim H, Bandyopadhyay A, Korzeniewski K, Ling Sia C, Dhindsa S, Chaudhuri A (2010) Insulin suppresses endotoxin-induced oxidative, nitrosative, and inflammatory stress in humans Diabetes Care 33:2416-2423 doi:10.2337/dc10-0929

Di Lorenzo A, Fernandez-Hernando C, Cirino G, Sessa WC (2009) Akt1 is critical for acute inflammation and histamine-mediated vascular leakage Proc Natl Acad Sci U S A 106:14552-14557 doi:10.1073/pnas.0904073106

Dong C, Davis RJ, Flavell RA (2002) MAP kinases in the immune response Annu Rev Immunol 20:55-72 doi:10.1146/annurev.immunol.20.091301.131133

Endo K, Takino T, Miyamori H, Kinsen H, Yoshizaki T, Furukawa M, Sato H (2003) Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration J Biol Chem 278:40764-40770 doi:10.1074/jbc.M306736200

Genini S et al. (2011) Strengthening insights into host responses to mastitis infection in ruminants by combining heterogeneous microarray data sources BMC Genom 12:225 doi:10.1186/1471-2164-12-225

Ghanim H et al. (2008) Acute modulation of toll-like receptors by insulin Diabetes Care 31:1827-1831 doi:10.2337/dc08-0561

Gholizadeh-Ghaleh Aziz S, Pashaei-Asl F, Fardyazar Z, Pashaiasl M (2016) Isolation, Characterization, Cryopreservation of Human Amniotic Stem Cells and Differentiation to Osteogenic and Adipogenic Cells PLoS One 11:e0158281 doi:10.1371/journal.pone.0158281

Gudkov AV, Gurova KV, Komarova EA (2011) Inflammation and p53: A Tale of Two Stresses Genes Cancer 2:503-516 doi:10.1177/1947601911409747

Hagiwara S, Mori K, Nagahata H (2016) Predictors of fatal outcomes resulting from acute Escherichia coli mastitis in dairy cows J Vet Med Sci 78:905-908

He X, Wei Z, Zhou E, Chen L, Kou J, Wang J, Yang Z (2015) Baicalein attenuates inflammatory responses by suppressing TLR4 mediated NF-kappaB and MAPK signaling pathways in LPS-induced mastitis in mice Int Immunopharmacol 28:470-476 doi:1/0.1016j.intimp.2015.07.012

Hogan J, Larry Smith K (2003) Coliform mastitis Vet Res 34:507-519 doi:10.1051/vetres:2003022

Hogeveen H, Huijps K, Lam TJ (2011) Economic aspects of mastitis: new developments N Z Vet J 59:16-23 doi:10.1080/00480169.2011.547165

Kanehisa M (2008) KEGG. Metabolic database. doi:http://www.genome.ad.jp/kegg/

Kargarfard F, Sami A, Ebrahimie E (2015) Knowledge discovery and sequence-based prediction of pandemic influenza using an integrated classification and association rule mining) CBA) algorithm J Biomed Inform 57:181-188 doi:10.1016/j.jbi.2015.07.018

Kobayashi T, Masumoto J, Tada T, Nomiyama T, Hongo K, Nakayama J (2007) Prognostic significance of the immunohistochemical staining of cleaved caspase-3, an activated form of caspase-3, in gliomas Clin Cancer Res 13:3868-3874 doi:10.1158/1078-0432.CCR-06-2730

Lamb DJ, Modjtahedi H, Plant NJ, Ferns GA (2004) EGF mediates monocyte chemotaxis and macrophage proliferation and EGF receptor is expressed in atherosclerotic plaques Atherosclerosis 176:21-26 doi:10.1016/j.atherosclerosis.2004.04.012

Lee HM, Ciancio SG, Tuter G, Ryan ME, Komaroff E, Golub LM (2004) Subantimicrobial dose doxycycline efficacy as a matrix metalloproteinase inhibitor in chronic periodontitis patients is enhanced when combined with a non-steroidal anti-inflammatory drug J Periodontol 75:453-463 doi:10.1902/jop.2004.75.3.453

Lee WR et al. (2013) Effects of chimeric decoy oligodeoxynucleotide in the regulation of transcription factors NF-kappaB and Sp1 in an animal model of atherosclerosis Basic Clin Pharmacol Toxicol 112:236-243 doi:10.1111/bcpt.12029

Lewis JD (2011) The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease Gastroenterology 140:1817-1826 e1812 doi:10.1053/j.gastro.2010.11058.

Li D et al. (2013) Emodin ameliorates lipopolysaccharide-induced mastitis in mice by inhibiting activation of NF-kappaB and MAPKs signal pathways Eur J Pharmacol 705:79-85 doi:10.1016/j.ejphar.2013.02.021

Malarstig A, Eriksson P, Hamsten A, Lindahl B ,Wallentin L, Siegbahn A (2008) Raised interleukin-10 is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome Heart 94:724-729 doi:10.1136/hrt.2007.119271

Manicone AM, McGuire JK (2008) Matrix metalloproteinases as modulators of inflammation Semin Cell Dev Biol 19:34-41 doi:10.1016/j.semcdb.2007.07.003

McGuire JK, Li Q, Parks WC (2003) Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium Am J Pathol 1843-162:1831 doi:10.1016/S0002-9440(10)64318-0

Mimoso C, Lee DD, Zavadil J, Tomic-Canic M, Blumenberg M (2014) Analysis and meta-analysis of transcriptional profiling in human epidermis Methods Mol Biol 1195:61-97 doi:10.1007/7651_2013_60

Minuti A et al (2015) .Acute mammary and liver transcriptome responses after an intramammary Escherichia coli lipopolysaccharide challenge in postpartal dairy cows Physiol Rep 3 doi:10.14814/phy2.12388

Mizia-Stec K, Gasior Z, Zahorska-Markiewicz B, Janowska J, Szulc A ,Jastrzebska-Maj E, Kobielusz-Gembala I (2003) Serum tumour necrosis factor-alpha, interleukin-2 and interleukin-10 activation in stable angina and acute coronary syndromes Coron Artery Dis 14:431-438 doi:10.1097/01.mca.0000085707.34267.70

Mohammadi A, Saraee MH, Salehi M (2011) Identification of disease-causing genes using microarray data mining and Gene Ontology BMC Med Genomics 4:12 doi:10.1186/1755-8794-4-12

Nikitin A, Egorov S, Daraselia N, Mazo I (2003) Pathway studio--the analysis and navigation of molecular networks Bioinformatics 19:2155-2157

Pan MR, Hung WC (2002) Nonsteroidal anti-inflammatory drugs inhibit matrix metalloproteinase-2 via suppression of the ERK/Sp1-mediated transcription J Biol Chem 277:32775-32780 doi:10.1074/jbc.M202334200

Panahi B, Mohammadi SA, Ebrahimi Khaksefidi R, Fallah Mehrabadi J, Ebrahimie E (2015) Genome-wide analysis of alternative splicing events in Hordeum vulgare: Highlighting retention of intron-based splicing and its possible function through network analysis FEBS Lett 589:3564-3575 doi:10.1016/j.febslet.2015.09.023

Papageorgiou AP, Heymans S (2012) Interactions between the extracellular matrix and inflammation during viral myocarditis Immunobiology 217:503-510 doi:10.1016/j.imbio.2011.07.011

Parks WC, Wilson CL ,Lopez-Boado YS (2004) Matrix metalloproteinases as modulators of inflammation and innate immunity Nat Rev Immunol 4:617-629 doi:10.1038/nri1418

Pathi S, Jutooru I, Chadalapaka G, Nair V, Lee SO, Safe S (2012) Aspirin inhibits colon cancer cell and tumor growth and downregulates specificity protein (Sp) transcription factors PLoS One 7:e48208 doi:10.1371/journal.pone.0048208

Pathi S, Li X, Safe S (2014) Tolfenamic acid inhibits colon cancer cell and tumor growth and induces degradation of specificity protein (Sp) transcription factors Mol Carcinog 53 Suppl 1:E53-61 doi:10.1002/mc.22010

Portt L, Norman G, Clapp C, Greenwood M, Greenwood MT (2011) Anti-apoptosis and cell survival: a review Biochim Biophys Acta 1813:238-259 doi:10.1016/j.bbamcr.2010.10.010

Ramasamy A, Mondry A, Holmes CC, Altman DG (2008) Key issues in conducting a meta-analysis of gene expression microarray datasets PLoS Med 5:e184 doi:10.1371/journal.pmed.0050184

Rech J et al. (2016) Prediction of disease relapses by multibiomarker disease activity and autoantibody status in patients with rheumatoid arthritis on tapering DMARD treatment Ann Rheum Dis 75:1637-1644 doi:10.1136/annrheumdis-2015-207900

Rinaldi M, Li RW, Capuco AV (2010) Mastitis associated transcriptomic disruptions in cattle Vet Immunol Immunopathol 138:267-279 doi:10.1016/j.vetimm.2010.10.005

Shafi S, Lamb D, Modjtahedi H, Ferns G (2010) Periadventitial delivery of anti-EGF receptor antibody inhibits neointimal macrophage accumulation after angioplasty in a hypercholesterolaemic rabbit Int J Exp Pathol 91:224-234 doi:10.1111/j.1365-2613.2009.00700.x

Sharifi S, Pakdel A, Ebrahimi M, Reecy JM, Fazeli Farsani S, Ebrahimie E (2018) Integration of machine learning and meta-analysis identifies the transcriptomic bio-signature of mastitis disease in cattle PLoS One 13:e0191227 doi:10.1371/journal.pone.0191227

Sipka A, Klaessig S, Duhamel GE, Swinkels J, Rainard P, Schukken Y (2014) Impact of intramammary treatment on gene expression profiles in bovine Escherichia coli mastitis PLoS One 9:e85579 doi:10.1371/journal.pone.0085579

Soehnlein O et al. (2010) Anesthesia aggravates lung damage and precipitates hypotension in endotoxemic sheep Shock 34:412-419 doi:10.1097/SHK.0b013e3181d8e4f5

Song X, Guo M, Wang T, Wang W, Cao Y, Zhang N (2014) Geniposide inhibited lipopolysaccharide-induced apoptosis by modulating TLR4 and apoptosis-related factors in mouse mammary glands Life Sci 119:9-17 doi:10.1016/j.lfs.2014.10.006

Subramanian A et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles Proc Natl Acad Sci U S A 102:15545-15550 doi:10.1073/pnas.0506580102

Tan KW et al. (2013) Neutrophils contribute to inflammatory lymphangiogenesis by increasing VEGF-A bioavailability and secreting VEGF-D Blood 122:3666-3677 doi:10.1182/blood-2012-11-466532

Too LK, Ball HJ, McGregor IS, Hunt NH (2014) The pro-inflammatory cytokine interferon-gamma is an important driver of neuropathology and behavioural sequelae in experimental pneumococcal meningitis Brain Behav Immun 40:252-268 doi:10.1016/j.bbi.2014.02.020

Turner MD, Nedjai B, Tara Hurst T, Pennington DJ (2014) Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease Biochim Biophys Acta 1843:2563-2582

Wei D ,Wang L, He Y, Xiong HQ, Abbruzzese JL, Xie K (2004) Celecoxib inhibits vascular endothelial growth factor expression in and reduces angiogenesis and metastasis of human pancreatic cancer via suppression of Sp1 transcription factor activity Cancer Res 642038-2030:

Younis S, Javed Q, Blumenberg M (2016) Meta-Analysis of Transcriptional Responses to Mastitis-Causing Escherichia coli PLoS One 11:e0148562 doi:10.1371/journal.pone.0148562

Zhang WR et al. (2015) Plasma IL-6 and IL-10 Concentrations Predict AKI and Long-Term Mortality in Adults after Cardiac Surgery J Am Soc Nephrol 26:3123-3132 doi:10.1681/ASN.2014080764