Gene Co-Expression Reveals Pathways by which Chicken Spleen Responds to Avian Pathogenic *Escherichia coli*

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Summary and Implications

This study identified genetic pathways that respond to avian pathogenic *Escherichia coli* (APEC) infection using shared gene expression patterns (co-expression) in chicken splenic RNA-sequencing. Ten co-expressed gene clusters were significantly correlated with APEC challenge and/or bacterial load. The positively correlated clusters included genes that function in cell division and immune signaling and suggest immune activation in the spleen after APEC challenge. Future research could study these pathways as targets to improve chicken resistance to APEC, and if verified, they may be useful in developing strategies to enhance natural resistance to APEC.

Introduction

Colibacillosis is one of the most common and detrimental bacterial diseases in commercial poultry worldwide. These APEC infections reduce poultry growth and reproductive performance, and can cause high mortality. Although vaccines exist, the diversity of APEC serotypes limits vaccine efficacy. Increasing host natural resistance to APEC could be more protective, but requires better understanding of chicken responses to APEC. This study used global gene expression in chicken spleen, an important immune tissue, to detect shared gene expression patterns (co-expression), predict gene functions, and identify genetic pathways that respond to APEC.

Materials and Methods

Animals and Bacterial Challenge: The F_1 chicks (from crosses of a disease-resistant Fayoumi line and a disease-susceptible broiler line) were divided into APEC challenged and non-challenged groups. At 14 days of age, the challenged group was injected into the right air sac with APEC O1:K1:H7; the non-challenged group received sterile saline. At 1 or 2 days post infection, birds were euthanized and spleen, lung and liver harvested.

Bacteriology: Homogenized tissue samples (in 10-fold serial dilutions) were plated on MacConkey agar and incubated overnight at 37 °C. Resulting APEC colonies were counted to determine level of bacterial load in each sample.

RNA-sequencing: Splenic mRNA was used to generate cDNA libraries
(n = 5-6 libraries/cross/challenge status/day post infection), which were sequenced. Short sequence reads were mapped onto the chicken genome and the number of expressed sequences per gene was counted.

Co-expression Analysis: Read counts were used in Weighted Co-expression Network Analysis (WGCNA) to identify clusters of genes that shared gene expression patterns. Pearson correlations between these co-expressed gene clusters and sample traits (challenge status and bacterial load) were generated and assessed for significance (p-value < 0.05). Potential functions for significant gene clusters were identified by a Gene Ontology (GO) term overrepresentation test (PANTHER; FDR-adjusted p-value < 0.05).

Results and Discussion

The WGCNA identified 17 clusters of co-expressed genes in chicken spleen, of which 10 clusters were significantly correlated with APEC challenge status and/or bacterial load in each tissue (Table 1). The highest correlations were detected for cluster 3 and 4 (positive) and cluster 9 and 10 (negative). Cluster 4 contained many genes involved in interconnected aspects of the immune response, (Table 1, Figure 1) The increased expression of immune genes (such as *CCL1*, *CCL4*, and *TLR1B*) in the APEC challenged group therefore likely reflects signaling for immune activation in the spleen. Cluster 3 also suggests immune cell proliferation under APEC infection, with overrepresentation of genes related to cell proliferation (Table 1). For example, slight but significant increases in expression of transcription factors (*E2F3* and *E2F7*), regulatory kinases (*CDK1* and *PLK1*) and chromosome maintenance genes (*MCM2*, *MCM5*, and *MCM6*; Figure 2) could all affect progression of the cell cycle. Overall, co-expression revealed mechanisms of early immune activation in the spleen and modulation of these pathways provide routes to improve resistance to APEC.

Acknowledgments

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Table 1. Correlation of ten co-expressed gene clusters with APEC challenge status and/or bacterial load[1].

Gene Cluster	Challenge Status[2]	Bacterial Load[3]			Top Overrepresented GO Terms[4]
		Spleen	Lung	Liver	
1	NS	0.42	0.34	NS	response to stimulus
2	0.52	0.56	0.38	NS	ribosomal subunit export from nucleus, protein targeting to ER
3	0.81	0.66	0.63	0.51	chromosome segregation, DNA replication initiation
4	0.70	0.77	0.55	0.38	negative regulation of T cell activation, T cell proliferation
5	0.61	0.57	0.4	0.38	---
6	0.41	NS	0.32	NS	---
7	0.32	0.43	0.35	NS	mitochondrial electron transport, ATP biosynthetic process
8	-0.43	-0.37	NS	NS	---
9	-0.75	-0.62	-0.47	-0.47	negative regulation of neuron differentiation, response to lipid
10	-0.85	-0.79	-0.60	-0.48	response to fibroblast growth factor, cell junction organization

[1]Pearson correlation values shown for significant gene clusters (p-value < 0.05). Not significant (NS).
[2]APEC challenged vs. non-challenged.
[3]Bacterial loads measured as log transformed colony forming units per gram of tissue ($\log_{10}$(CFU/g)).
[4]Subset of top overrepresented (FDR-adjusted p-value < 0.05) GO-SLIM biological process terms for each gene cluster.

Figure 1. Immune genes involved in toll-like receptor and cytokine signaling correlate with APEC challenge. Relationships between GO "immune response" genes from cluster 4 are shown. Text color indicates genes up-regulated (red) or down-regulated (blue) by APEC challenge.

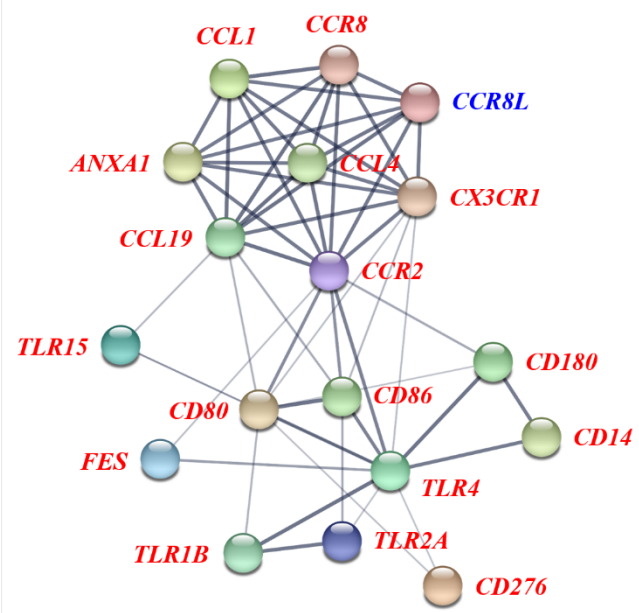

Figure 2. Mediators and regulators of the cell cycle also correlate with APEC challenge. Relationships between GO "cell cycle" genes from the cluster 3 are shown. Text color indicates genes up-regulated (red) or down-regulated (blue) by APEC challenge.