Integration of Host and Virus Gene Expression for Chickens Response to Avian Leukosis Virus Challenge

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Summary and Implications

The tumorigenesis caused by avian leukosis virus (ALV) occurs through a complex regulatory architecture. , We applied a novel methodology to jointly analyze four different types of datasets: mRNA expression, the associated regulatory factors of miRNA and lncRNA, and ALV gene expression. The pathway analysis shows that combined analysis produced more informative results than single data-type analyses, and the co-expression network analysis found key elements involved in the host-pathogen interaction. The integration of multiple data sources yielded deeper insights into the pathogenesis of the virus and the host immune response.

Introduction

Among the 6 subgroups of ALVs, subgroup J (ALV-J) infection causes significant economic loss due to increased mortality and decreased production. However, the pathogenic mechanism of host immunosuppression caused by ALV-J is poorly understood due to the complex host-pathogen interactions. Performing sequencing of host mRNA, miRNA, lncRNA, and viral gene expression, and combining these sequencing results for analyses, we hope to gain novel insights into the complex interaction.

Materials and Methods

A total of six spleen samples (3 control and 3 challenged) were collected 40 days post injection from specific pathogen-free white Leghorn chickens that have either been challenged with ALV-J on the first day of age (challenged) or injected with saline (control). Total RNA was isolated from the spleen samples, and mRNA, miRNA, and lncRNA were sequenced simultaneously with Illumina HiSeq 2000. The sequence data was mapped to the chicken reference genome (*Gallus gallus* 4.0) with TopHat2 and differentially expressed (DE) transcripts were identified with edgeR.

DE miRNA and lncRNA were used to select targeted DE mRNA to form the miRNA and lncRNA groups, respectively. The virus group was formed by identifying DE mRNA that were correlated with ALV genes. The gene lists from all 4 groups (mRNA, miRNA, lncRNA, and virus) were loaded into Ingenuity Pathway Analysis (IPA) for identification of enriched pathways. The gene expression levels of mRNA, miRNA, lncRNA, and virus were combined for the drawing of a co-expression network based on correlation.

Results and Discussion

The challenge of ALV-J resulted in 864 mRNAs to be DE, of which, 260 were targeted by 7 DE miRNAs, 390 were targeted by 14 DE lncRNAs, and 380 were correlated with 8 ALV genes. There were a total of 34 shared DE genes among the miRNA, lncRNA, and virus groups. The IPA analysis showed consistent predicted regulation of enriched pathways (Figure 1). The IPA analysis also demonstrated that DE mRNA alone gave the least information result with only cAMP-mediated signaling pathway having a predicted down-regulation.

There were 34 distinct co-expression networks that contained 5 or more nodes, of which, 2 networks contained nodes directly related to ALV-J stimulation and immune response. In one network, ALV gene *env* had direct interaction with 2 miRNAs and 8 mRNA (Figure 2a). In the other network, a lncRNA (LOC_009715) is the central hub in a network that contained 9 mRNA, including IRF3, which is a critical gene in innate immunity mediated by interferons (Figure 2b).

In summary, the combined analysis of different sequencing datasets provided clearer results in pathway analysis and began to map out the complex interaction of ALV and host gene expression by predicting new gene targets that may be involved in the host-pathogen interaction.

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Figure 1. Heatmap comparing IPA pathway analysis of the 4 groups. The more orange the square in the heatmap, the greater the activity. In contrast, the more blue the square in the heatmap, the greater the inhibition.

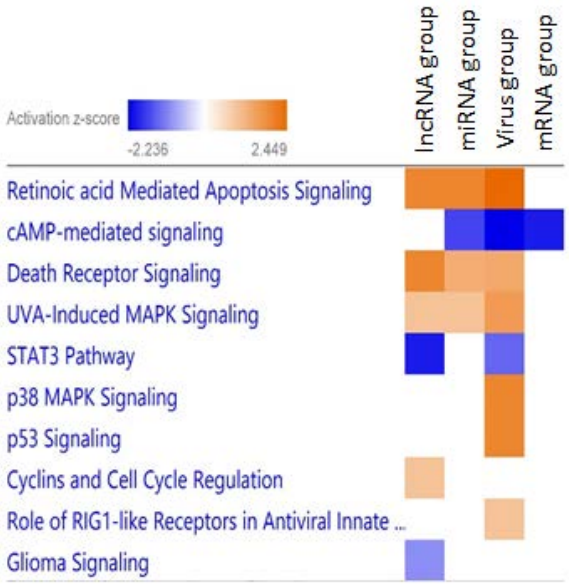

Figure 2. Co-expression networks of mRNA DEGs, DE miRNAs, DE lncRNAs and expressed viral genes. (A) Network has an ALV gene (env) directly interacting with 2 miRNAs (miR-7 and novel_51) and 8 protein-encoding genes. (B) Network has an lncRNA (LOC_009715) as the central regulator of 9 protein-encoding genes.