Virus Research 244 (2018) 147–152

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Transcription analysis of the interaction between chicken thymus and recombinant avian leukosis virus isolate FJ15HT0

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ARTICLE INFO

Keywords:
Ciliary neurotrophic factor
Transcriptome
Avian leukosis virus
Weight decrease

ABSTRACT

Avian leukosis virus (ALV) induces multiple avian tumors, growth decrease and immune suppression. Previously, a novel natural recombinant ALV isolate FJ15HT0 was proven to be associated with significant body weight decrease, immune suppression and lymphocytoma in infected SPF chickens. In order to uncover the interaction between virus and host, we compared differences in the transcriptomes of the thymuses from the mock chickens and simulated congenitally infected chickens at 5 days (d), 13d and 21d of age by RNA-seq analysis of the thymuses. Signaling pathways including cytokine–cytokine receptor interactions, peroxisome proliferator-activated receptor (PPAR) signaling pathway, Janus tyrosine kinase/signal transducers and activators of transcription (Jak-STAT) signaling pathway and fatty acid degradation were involved in the interaction between FJ15HT0 and SPF chickens. Interestingly, fold change of *ciliary neurotrophic factor receptor α* (*CNTFRα*) in infected donor collected from 2d to 21d showed a significant positive correlation with the corresponding expression of the viral *gp85* gene in thymuses (r = 0.656, $P < 0.01$) and in livers (r = 0.525, $P < 0.05$). It will provide new insights for the molecular pathogenesis of ALV infection.

1. Introduction

Avian leukosis viruses (ALVs), a group of oncogenic retroviruses, can be divided into 6 subgroups (A–E and J) that are associated with tumorigenesis, growth retardation and serious immunosuppression in chicken flocks (Fadly, 2003). The interaction between host and virus has drawn great attention. Using high-throughput techniques, such as next generation sequencing and microarray analysis, several *in vivo* or *in vitro* studies have identified differentially expressed genes or proteins from ALV infected birds and different cell types (Hang et al., 2014; Dai et al., 2016; Wang et al., 2013; Li et al., 2015). These previous studies, all of which show diverse results with different ALV subgroups and strains, have not yet revealed the pathogenic mechanism of ALV.

A novel naturally occurring recombinant ALV strain FJ15HT0 induced significant weight loss and immune suppression in simulated congenitally infected SPF chickens (Wu et al., 2017). To achieve new insights into the interaction between the host and this novel infectious organism, we analyzed the effect of FJ15HT0 infection on the expression of host factors *in vivo* at the RNA level using RNA-sequencing. Host gene expression profiles were significantly changed at the time points tested. Genes involved in cytokine–cytokine receptor interactions, PPAR signally pathway, Jak-STAT pathway and fatty acid degradation were differentially expressed in the thymus tissue of FJ15HT0 infected chickens. Notably, the *ciliary neurotrophic factor receptor α (CNTFRα)* gene was significantly correlated with fold change of *gp85* in infected thymuses and livers, accompanied by increased serum CNTF titer, demonstrating *CNTFRα* is involved in the interaction of host and FJ15HT0.

2. Methods

2.1. Virus

The novel ALV isolate FJ15HT0 was obtained from Chinese local chicken flock, with the gp85 gene from B subgroup and LTR from ALV-J. Previous research found that this isolate associated with body weight decrease, immune suppression and lymphocytoma in the liver and spleen tissues in experimental chickens (Wu et al., 2017).

2.2. Experimental groups

Five-days-old specific-pathogen-free (SPF) White Leghorn

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https://doi.org/10.1016/j.virusres.2017.11.018
Received 2 August 2017; Received in revised form 29 October 2017; Accepted 15 November 2017
Available online 21 November 2017
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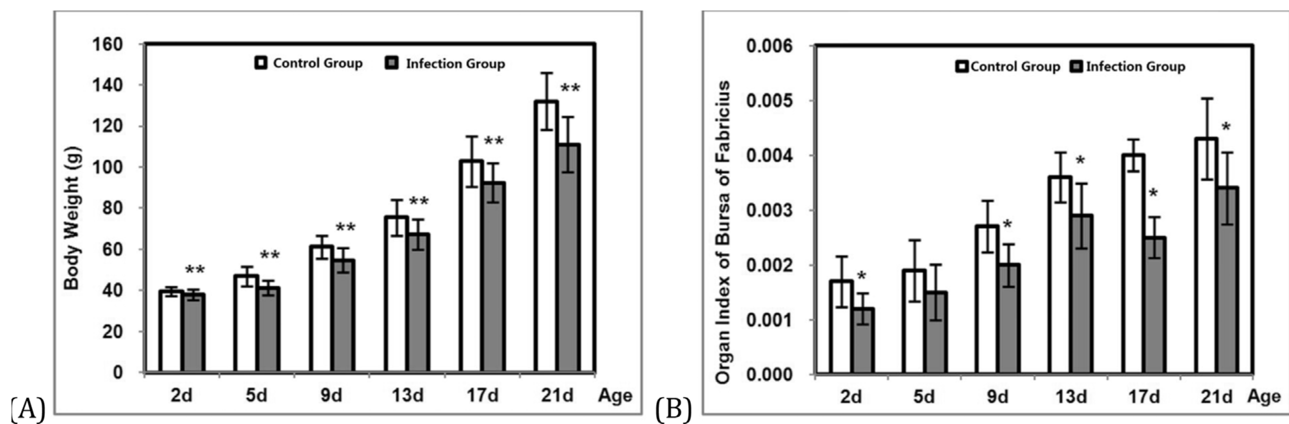

Fig. 1. Suppression of FJ15HT0 infection to the body weight (mean ± SE) (A) and the organ index of the bursa of Fabricius (mean ± SE) in the two groups (B). Significant differences between the two groups at $P < 0.05$ and $P < 0.01$ are denoted by "*" and "**", respectively.

embryonated chicken eggs (n = 96) were randomly divided into two groups with 48 eggs per group. Eggs in the infected group were inoculated *via* vitellicle inoculation on day 6 of embryonation with 6.3×10^6 tissue-culture infective dose ($TCID_{50}$) of the recombinant ALV isolate FJ15HT0, closely mimics the route of congenital infection. All eggs in control group were inoculated with DMEM in the same manner. Birds hatched from each of the two groups were raised separately in isolation feeding devices for 21d. The infection of inoculated SPF chickens was confirmed by shedding of the virus one day after hatching, determined by Avian Leukosis Virus Antigen Test Kit (IDEXX Laboratories) as p27 positive. Randomly, six donors per group were slaughtered at the age of 2d, 5d, 9d, 13d, 17d and 21d respectively. The body weight difference of the infected chickens were calculated by the following formula: Body weight difference = body weight of infected chicken (g) – Average body weight of control chickens at the same age (g). Samples of serum, thymus, bursa of Fabricius, spleen and liver were taken from each chickens. Each organ was weighted after dissecting connective tissue around and organ index was calculated by the following formula: Organ index = organ weight (g)/body weight (g).

2.3. RNA-seq and transcriptome analysis

On the age of 5d, 13d and 21d, three chickens per group were collected. Total RNA from uninfected and FJ15HT0 infected thymus tissue was subjected to RNA-seq and transcriptome analysis. Briefly, total RNA was extracted using PureLink RNA Mini Kit (Invitrogen) according to the manufacturer's instructions, quantified using NanoDrop ND-2000, and checked for RNA integrity with an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). RNA libraries were generated using TruSeq Stranded RNA Sample Prep Kit (Illumina) following the manufacturer's protocol, quantified with Qubit 2.0 Fluorometer (Invitrogen), examined for dot distribution with Agilent Bioanalyzer 2100, and subjected to sequencing using an Illumina Hiseq 2000. Acquired data were processed to obtain raw reads, which were then extracted for read mapping using TopHat (version 2.0.9) (Trapnell et al., 2009). Differentially expressed genes (DEGs) were identified using edger (Jingwen et al., 2015). The DEGs were subjected to DAVID (Database for Annotation, Visualization and Integrated Discovery) analysis to perform functional annotation based on the GO (Gene Ontology) database ($P < 0.05$), and pathway enrichment analysis based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) database ($P < 0.05$) (Huang et al., 2008).

2.4. Quantitative real-time PCR analysis for DEG and FJ15HT0 gp85 gene expression

RNA extraction and qRT-PCR for selected genes was performed as reported previously (Rawat and Mitra, 2011). The qRT-PCRs were performed using SYBR Premix ExTaq™ II (Perfect Real Time) (TAKARA, Japan) following the manufacturer's instructions. Reactions contained SYBR Green I qRT-PCR primer sets specific for chicken *IL7R*, *CCL19*, *FABP2*, *APOA1*, *IL2RB*, *IL15*, *AOX1*, *CNTFRα, SOCS3, GFAP, FABP4, ACSL3* and *gp85* gene of FJ15HT0 strain. The chicken *β-actin* and *GATPH* gene served as internal control. We performed qRT-PCR on an ABI7900HT, and each sample was run in triplicate. Gene expression levels were measured using the $2^{-\Delta\Delta Ct}$ method. Gene primer sequences are shown in S1 File.

2.5. ELISA

The samples of the serum collected from infected and control chickens were detected for the CNTF titer using an ELISA kit (Wuxi Donglin Sci & Tech Development Co.,Ltd. China). The D-value of the CNTF titer of the infected chickens were calculated by the following formula: D-value = CNTF titer of the infected chicken (pg/mL) – Average CNTF titer of control birds at the same age (pg/mL).

2.6. Statistical analysis

Gene expression was calculated relative to *β-actin* and *GADPH* using the comparative cycle time (Ct) method. All cross-level comparisons by one-way ANOVA method and correlation analysis using Spearman's rank correlation analysis method (positive correlation with absolute value > 0.15) were all two-sided, at a significance level of 0.05 and 0.01 using the SPSS statistical software package (version 19.0; SPSS Company, Chicago, IL, USA).

3. Results

3.1. Body weight suppression was correlated with changes in gp85 expression in the thymus

Six day old eggs were inoculated in the vitellicle with 6.3×10^6 $TCID_{50}$ FJ15HT0. The body weight ($P < 0.01$, Fig. 1A) and organ index of the bursa of Fabricius ($P < 0.05$, Fig. 1B) in the simulated congenitally infected birds was significantly suppressed, while the organ index of spleen, thymus and liver between the two groups showed no significant differences at the 6 time points tested ($P > 0.05$, data not shown). Expression of *gp85* in different tissues was measured using qRT-PCR at different time points. The correlation analysis showed that changes in *gp85* expression in infected tissues including bursa of Fabricius (r = −0.546), spleen (r = −0.502) and liver (r = −0.517), showed significant correlation with the corresponding body weight difference ($P < 0.05$). The fold change of the *gp85* in infected

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