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Short Communication

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Abstract

The tripartite motif containing 62 (TRIM62) is a host antiviral factor. It was demonstrated in our previous study that chicken TRIM62 significantly inhibited the replication of reticuloendotheliosis virus (REV). However, the effect of viral infection on the distribution of TRIM62 is still unknown. In the present study, we tested and evaluated the tissue distribution of TRIM62 and the REV infection correlativity. We found that the distribution and mRNA expression of TRIM62 were first increased and then decreased upon REV infection. The decrease of TRIM62 was mostly in the spleen. The results suggest REV inhibited the antiviral activity of TRIM62 by decreasing its expression.

Keywords: tripartite motif containing 62 (TRIM62), reticuloendotheliosis virus (REV), tissue distribution, correlativity

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Introduction

Reticuloendotheliosis virus (REV) is an avian retrovirus that causes immunosuppression. REV is one of the most important and common pathogens in chickens. The immune state of the host is closely related to host resistance. Host cells express specific proteins, referred to as restriction factors, to interfere with the replication of retroviruses (Uchil *et al*., 2008). Host innate immune factors play a major role in protective anti-infective immunity (Weidenbusch *et al*., 2017).

The tripartite motif (TRIM) family represents a new class of anti-retroviral proteins involved in innate immunity (Nisole *et al*., 2005). As a new member of the tripartite motif (TRIM) protein family, tripartite motif containing 62 (TRIM62) has attracted much attention due to its crucial role in innate immune response (Cao *et al*., 2015; Yang *et al*., 2016). We have demonstrated that TRIM62 significantly inhibits the replication of avian retrovirus (REV and ALV-J) in vitro (Li *et al*., 2019; Li *et al*., 2020). However, little is known about the distribution of TRIM62 in chickens and far less is known about its dynamic changes after REV infection. In the present study, we tested and evaluated the tissue distribution of TRIM62 and the REV infection correlativity.

Materials and Methods

Cell, virus and chicken: The stock spleen necrosis virus (SNY) strain of REV (China strain: JX0927) was maintained in our laboratory. The $TCID_{50}$ of the virus was determined and DF-1 cells were cultured as described in a previous study (Zhu *et al*., 2019). Specified pathogen free (SPF) chicken embryos were purchased from Jinan Spafas Animal Inc., and hatched chickens were maintained at isolator. Each SPF chicken was inoculated with 4000 $TCID_{50}$ REV as previously reported (Dong *et al*., 2015). A total of 30 one day old SPF chickens were evenly divided into two groups. The chickens were culled every week, 3 chickens being taken from each group. All kinds of tissue were collected. This study was carried out in strict adherence to the recommendations in line with the Care and Use of Laboratory Animals of the Shandong Agricultural University. Ethical euthanasia of the chickens was performed by anesthesia. The anesthetic induction was performed using 846 Narcotic mixture (a combination of Jingsongling, haloperidol and dihydroetorphine, 20 mg/mL). Intramuscular injection of 0.2 mL per kg of body weight was given to each chicken.

Immunohistochemistry (IHC): To determine the tissue distribution of chickens of TRIM62 after REV infection at different times (7, 14, 21 and 28 days), immunohistochemistry was performed using anti-chicken TRIM62 monoclonal antibody incubated sections of tissue as described previously (Wang *et al*., 2018).

Quantitative real-time PCR (qRT-PCR): Total RNA from tissues (heart, liver, lungs, kidneys, brain, spleen, bursa of fabricius, proventriculus, and duodenum) was extracted using RNAprep Pure Tissue Kit and then reverse transcripted to cDNA (Li *et al*., 2020). The qRT-PCR was performed as for the previously described protocol to detect the mRNA expression of REV and TRIM62 with specific primers (Table 1) (Li *et al*., 2019). Statistical analysis was performed using SPSS 19.0 statistical software and the significances were determined at $p < 0.05$ (*).

Table 1 Primers used for quantitative reverse transcription-PCR

Gene target	Primer sequence	Fragment size (bp)
TRIM62	Forward: TACTGGGAGGTGGTGGTGTC Reverse: CGTCGGCGTTGTAGAAGATG	246
REV (env)	Forward: TTGTTGAAGGCAAGCATCAG Reverse: GAGGATAGCATCTGCCCTTT	330
GADPH	Forward: GAACATCATCCCAGCGTCCA Reverse: CGGCAGGTCAGGTCAACAAC	132

Results and Discussion

In order to probe the distribution of TRIM62 in vivo, we detected the expression of TRIM62 by IHC in various tissues at different times after infection. The results of IHC showed that TRIM62 was ubiquitously expressed in various tissues and highly expressed in the spleen, brain, bursa of fabricius and thymus of uninfected chickens. TRIM62 was mainly localized in epithelial cells. Otherwise, we found that the protein expression levels of TRIM62 was at first upregulated (14 days after infection) and then down regulated (28 days after infection) in most tissues after REV infection (part of results are shown in Figure 1), which was consistent with the results we observed in vitro (Li *et al*., 2020).

To further explore the dynamic changes of TRIM62 expression after REV challenging, the expression of TRIM62 at mRNA level and the relative abundance of REV were determined in various tissues. The trend of TRIM62 mRNA expression was similar to that of protein expression. After REV infection, the TRIM62 mRNA expression first increased and then decreased with the increase of REV replication. At 28 days after infection, the abundance of REV mRNA significantly increased in various tissues over that at 14 days ($p<0.01$) (Figure 2A). Upon REV infection, the mRNA expression of TRIM62 significantly decreased especially in the spleen, liver, kidneys, heart, lungs and proventriculus ($p<0.001$) (Figure 2B). The decrease of TRIM62 was mostly in the spleen (Figure 2B). These

results suggest that effect of REV infection on TRIM62 was mostly in the immune organs.

In this study, we found that TRIM62 expression at first increased and then decreased in various tissues of the infected chickens upon REV infection. The down-regulation of TRIM62 was mostly in the spleen. The TRIM62 as a member of the TRIM family has been found to participate in the antiviral process (Li *et al*., 2019; Li *et al*., 2020). Our results indicated that the host was at first active in the antiviral effect of TRIM62 after REV infection and then REV inhibited the antiviral activity of TRIM62 by decreasing its expression. Further studies are needed to understand the mechanism.

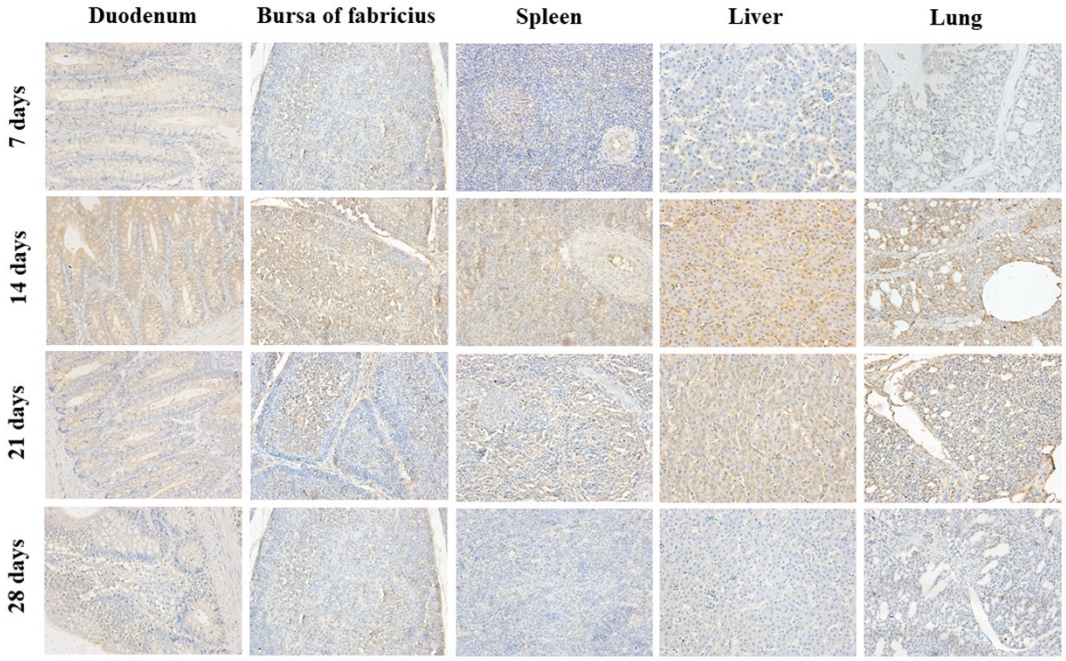

Figure 1 The analysis of TRIM62 expression in various tissues at different times after REV infection by IHC assay. Positive cells are shown in brown. (200×)

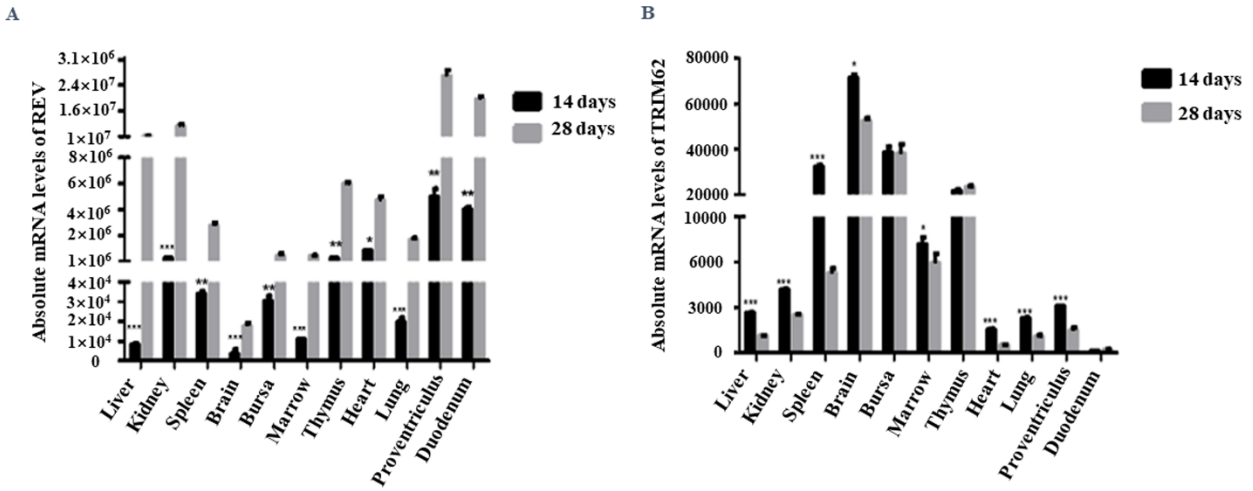

Figure 2 The mRNA levels of REV and TRIM62 were analysed by qRT-PCR. (A) REV expression levels were significantly increased in different tissues at 28 days after REV infection over that at 14 days. (B) TRIM62 expression was down-regulated significantly in different tissues at 28 days after REV infection. *, p<0.05, **, p<0.01, ***, p<0.001

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