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Molecular cloning and expression analysis of *TRAF3* in chicken

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ABSTRACT. Tumor necrosis factor receptor-associated factor 3 (TRAF3) is a crucial regulator that suppresses c-Jun N-terminal kinase and non-canonical nuclear factor-κB signaling, but facilitates type I interferon production. To determine TRAF3 function in innate immune responses among birds, particularly chicken, we cloned and characterized the chicken TRAF3 gene (*chTRAF3*) and detected its tissue expression profile in chicken. We also detected the differential expression of *chTRAF3* and its downstream gene interferon-β (*IFN-β*) upon different stimuli in primary chicken embryo fibroblast cells. Two *chTRAF3* gene products, *chTRAF3-1* and *chTRAF3-2*, can be produced by alternative splicing. The full-length coding sequence of *chTRAF3* (*chTRAF3-1*) was 1704 base pairs and encoded a protein of 567 amino acids with high identity to TRAF3 homologs from mammals and other birds. The deduced amino acid sequence showed typical characteristics of TRAFs, with a RING finger domain, 2 zf-TRAF motifs, and a MATH domain. Quantitative real-time polymerase chain reaction analysis revealed broad expression of *chTRAF3* in all detected tissues, with abundant expression in the spleen, thymus, lung, and

small intestine. Expression of *chTRAF3* was significantly upregulated in a time- and concentration-dependent manner in chicken embryo fibroblast cells challenged with poly I:C or poly dA-dT. Furthermore, *chTRAF3* and *IFN-β* mRNA expression from chicken embryo fibroblast cells challenged with Newcastle disease virus F48E9 suffered intense suppression compared with Newcastle disease virus Mukteswar infection. Our results indicate that *chTRAF3* plays important roles in defending against both RNA and DNA virus infection.

Key words: Chicken; Cloning; Gene expression; Newcastle disease virus; Tumor necrosis factor receptor-associated factor 3