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ENHANCEMENT OF GENE EXPRESSION BY MAREK'S DISEASE VIRUS
HOMOLOGUE OF THE HERPES SIMPLEX VIRUS-1 ICP4

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Marek's disease virus (MDV) homologue of the herpes simplex virus-1-ICP4 (MDV ICP4) is a candidate of an important activator of MDV genes. The aim of this study is to show the evidence for activation of virus genes by MDV ICP4 and the existence of the cells expressing MDV ICP4 in the tissues of MDV-infected chickens.

Although Marek's disease (MD) tumor cell line, MDCC-MSB1 (MSB-1) contains multiple copies of MDV genomes, the transcriptin of MDV DNA occurs along limited portions of the viral genome. To observe the effect of MDV ICP4 on the expression of viral genes, I transfected MSB-1 tumor cells with the plasmid including a coding region of MDV ICP4 using a cationic liposome. As carriers for intranuclear transport, high mobility group-1 and -2 proteins were bound to the plasmid DNA before forming liposomes. I detected transcripts from the plasmid 2 hr after transfection by quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) analysis. I also detected abundant transcripts of MDV ICP4 from MDV genomes in the MSB-1 cells (endogenous ICP4) 2-96 hr after transfection. These findings suggested that expression of the introduced MDV ICP4 gene enhanced the expression of the endogenous MDV ICP4. On the other hand, transcripts of MDV DNA polymerase were not detected in transfected MSB-1 cells and quantitative PCR analysis for virus genome DNA indicated no significant alteration of copy number of the virus genome in transfected MSB-1 cells, suggesting that viral replication of MDV requires more than turning on the MDV ICP4 gene. As an important indicator of MD-infected cells in MDV-infected tissues, enhancement of expression of phosphoprotein pp38 (pp38) gene was also studied. For the measurement of promoter/enhancer activity, the promoter/enhancer element of pp38 was identified by cDNA sequencing, primer extension analysis and by open reading frame (ORF) prediction by analysis of recombinant protein synthesized from the ORF. The results showed that the promoter of pp38 exists 15-19 bases upstream from the 5'-end of the cDNA and that the enhancer sequences include ICP4, Sp-1 and Oct-1 binding elements. I constructed the plasmid pGV-pp which included the promoter/enhancer fragment of pp38 gene just upstream of the luciferase gene as a reporter gene and transfected into MSB-1 cells. Expression of the luciferase under the control of the promoter/enhancer of pp38 gene was enhanced when the cells were co-transfected with the MDV ICP4-expressing plasmid. These findings suggest that MDV ICP4 upregulates the enhancer of pp38.

Transfection experiments using the MDV ICP4-expressing plasmid suggest that the expression of MDV ICP4 preceded the expression of early or late genes. Thus a variety stage of MDV infection may be detected using MDV ICP4 as an indicator. To observe the MDV ICP4-expressing cells *in vivo*, I showed transcripts of the MDV ICP4 on paraformaldehyde-fixed and paraffin embedded cells by *in situ* hybridization (ISH). A cRNA-probe synthesized from the plasmid containing a 0.55 kb DNA fragment which locates on 1 kb upstream of MDV ICP4-ORF was selected as the probe for the MDV ICP4 transcripts, as it hybridized to full length transcripts on Northern blot. Using the digoxigenin-labeled 0.55 kb cRNA probe, the MDV ICP4 transcripts were detected in 92% of lytically-infected chicken embryo fibroblasts (CEF) and in 0.35% of MSB-1. No signal was observed on uninfected CEF. By this ISH method, I detected the MDV ICP4 transcripts in the feather follicle epithelium (FFE), lymphoid cells in the liver, kidneys and nerve of infected chickens. The MDV ICP4 transcripts were also detected in the FFE, nerve and lymphocytic foci of liver and kidney by RT-PCR.

In conclusion, MDV ICP4 is suggested to activate some MDV genes, while infection does not proceed to the lytic stage with MDV ICP4 alone. And I detected the MDV ICP4 transcripts in the lymphoid cells of MDV-infected chickens by the ISH method. The application of the ISH method on the observation of the expression of viral transactivators may resulted in significant advances in understanding the mechanisms for tumorigenesis by MDV.

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