Transcriptional analysis of Marek's disease virus (MDV) genes in MDV-transformed lymphoblastoid cells without activated cells

Masahiro Ui

Laboratory of Radiation Biology,
Department of Environmental Veterinary Sciences,
School of Veterinary Medicine,
Hokkaido University, Sapporo 060, Japan

Marek's disease virus (MDV) induces malignant lymphoma in chickens. Lymphoblastoid cell lines were established from embryonic lymphocytes infected *in vitro* with MDV as well as MDV-induced tumors. The majority of MDV-transformed lymphoblastoid cells are latently infected with MDV. The cells in which viral genes are limitedly expressed are referred to as MDV-transformed cells, including nonactivated cells. Analysis of the viral genes responsible for the latency or the maintenance of transformation has been carried out by a method characterizing mRNAs expressed in MDV-transformed cells. Although the molecular mechanisms are not clear, some cells spontaneously enter to the lytic cycle of MDV and are called MDV-activated cells. The presence of MDV-activated cells among MDV-transformed ones usually makes it difficult to analyze tumor- or latency-associated transcripts because of the quantitative and qualitative multiplicity of transcription of MDV-transformed cells.

Nonactivated MDV-transformed cells were prepared by simply reducing their number and subjected to analysis. Contamination by activated cells was confirmed to be less than 0.01% by immunohistochemically detecting a major MDV early antigen, pp38. This result made it possible to carry out reverse transcriptase-polymerase chain reaction (RT-PCR) with Southern-blot hybridization on the templates extracted from one thousand MDV-transformed cells. In six lines of MDV-transformed cells, RT-PCR products corresponding to ICP27, pol, US3, A41, gA, gB, TK and UL50 genes expressing during the lytic cycle were not detected in addition to the pp38 gene, but RT-PCR products corresponding to VP16, SORF2 and pp14 were detected in MSB-1, in MSB-1 and MOGA-2, and in RPL-1, respectively. A 0.7 kbp RT-PCR product located in the MDV ICP4 ORF was detected in all MDV-transformed cell lines, but not in an REV-transformed cell line. Therefore, the 0.7 kbp RT-PCR product was regarded as a novel transcript of MDV associated with the latency or the maintenance of transformation of the MDV-transformed cells. The 0.7 kbp RT-PCR product was mapped to both 5'- and 3'- end regions of MDV ICP4 ORF. From the distance between two regions of the 0.7 kbp RT-PCR product, 3,607 bases were suggested to be spliced out. No pp14 or A41 genes, which were reported to be expressed in both lytic and latent states, were transcribed in the cell populations of nonactivated MDV-transformed cells.

In conclusion, (i) a cell population of nonactivated MDV-transformed cells could be efficiently prepared by immunohistochemically detecting pp38 as an indication of activation, (ii) RT-PCR-Southern blot analysis of MDV genes collected from 1,000 cells revealed that a transcript common to RPL-1, HPRS-1, MOGA-1, MOGA-2, MSB-1 and MTB-1 was expressed.