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人類 JC 病毒基因調控之研究

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計畫主持人：王梅林

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ABSTRACT

Human polyomavirus, JC virus, may cause a fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), of the central nervous system mostly in immunodeficiency patients. Moreover, JC virus may also be involved in human tumorigenesis. Most people are infected by the virus without clinical symptoms. The archetypal JC virus containing a single copy of the promoter and enhancer in the transcriptional control region was found in normal population. However, the rearranged JC virus containing deletions and/or duplications in the transcriptional control region was frequently found in PML patients. Therefore, it is speculated that different genotypes of the virus may correlate with replication activity. During past years, several genotypes, Taiwan-1 to Taiwan-12, of JC virus were isolated from patients with autoimmune diseases and renal transplantation in our laboratory. The purpose of this research proposal is to further investigate the biological activity of the different genotypes of JC virus. Transcription activities of the various genotypes were analyzed using JCV genomic cloning and transfection into human neuroblastoma cells (IMR-32). The Results demonstrated that TW-4 and TW-12 JCV genomes showed the strongest replication activity after transfection and determined by Western blot using anti-VP1 antiserum. TW5 and TW-11 did not show any replication activity. Virions with various TCR will be further isolated and used to infect human neuroblastoma cells for comparison of various TCR activities.

INTRODUCTION

JCV strains fall into two groups, designated archetypal and rearranged, based on the structure of their transcriptional control regions (TCRs). The TCR of archetype JCV contains a single copy of the promoter and enhancer, while the rearranged strains contain deletions and duplications in this region (3, 31, 32). The archetype strains are believed to be the viruses that spread throughout the population and establish persistent infections, as they can be isolated from both normal individuals and immunocompromised patients (1, 13, 17, 23, 33). Rearranged strains of the virus have been isolated from brain, kidney, and lymphocytes of PML patients, as well as from brain, CSF, and lymphocytes of nonaffected individuals (3, 5, 7, 28, 30). The duplicated regions include the binding sites for a variety of transcription factors. Thus, it is believed that these rearrangements lead to altered transcription patterns of the early late regions, which could affect the level of viral replication and ultimately cause disease.

PML is a neurodegenerative disease that is clearly caused by JCV infection (27). Cell destruction is thought to be carried out by the lytic replication of the virus in oligodendrocytes, which are myelin-producing cells in the brain. Currently, it is not known when during the course of natural infection or how the virus gets into the CNS. Replication of the virus is controlled primarily at the transcriptional level, and a great deal of effort has gone into defining the factors that seem to allow for tissue-specific replication of the virus. As discussed above, the rearrangements in the TCR are thought to contribute to the increased replication capacity of the virus in the brain (6).

Sequence variation in the TCR can alter the cellular host range (26) and may allow JCV to switch between states of lytic and latent infection. The cellular host range of JCV could depend upon a number of factors, three of which are cellular characteristics. Initially, a JC virion must come in contact and bind with a candidate-cell that has appropriate cellular membrane receptors (11, 16), such as sialyloligosaccharides (specifically glycoproteins with terminal (2-6)-linked sialic acid). In addition, the candidate-cell must have uptake mechanisms (54), such as endocytic clathrin-coated pits, that the JC virion can exploit for passage to the nucleus. Ultimately, however, effectual JCV transcription and replication relies on the presence of suitable nuclear DNA binding proteins (4, 20), such as those of nuclear factors-1 (NF-1) family (9). The NF-1 proteins bind multiple sites within the viral transcriptional control region, and growing evidence suggests that cell types capable of supporting JCV infection have upregulated levels of the NF-1 class D (22, 24). Host DNA binding proteins that are affected by extracellular stimuli may in turn alter viral activity (21). Of note, inherent cellular protein polymorphisms (12) could also affect all of the JCV/candidate-cell interactions, and pre-existing infections with other viruses could supply trans-activators of JCV transcription and replication (8, 10, 18, 29). However, the rearrangement of TCRs of JCV can affect the host cell range, lytic (late gene transcription) and latent (early gene transcription) infection of JCV. Therefore, it is an important issue in understanding the correlation between JCV gene regulation and cellular pathogenesis.

For the past few years we have been involved in the investigation of epidemiology and genotyping of human polyomaviruses in Taiwan. Four different strains, CY, Taiwan-1 (TW-1), Taiwan-2 (TW-2), and Taiwan-3 (TW-3) of JC V were found in pregnant individuals and autoimmune disease patients in Taiwan. More recently, various rearranged genotypes of JCV were found in renal transplantation patients. The new genotypes were

designated TW-4 through TW-12 with GenBank accession numbers AF218436 through AF218444. The TCRs of the Taiwan isolates contain deletions and/or tandem repeats. Variation on the TCRs may alter transcription activity. The various TCRs of the Taiwan isolates have been cloned into a replicative vector. Their biological activities will be investigated in this research proposal. If our proposal can be supported, the transcriptional activity of the various JCV promoters (TW-1~TW-12) will be analyzed. These fundamental informations may allow us to be better understanding the subtle mechanisms of JC virus infection and cellular pathology.

MATERIALS AND METHODS

Cell lines

Human neuroblastoma cell line (IMR32; CCRC-60014) was used for JCV genomic transfection and JCV infection. DMEM (Dulbecco's modified Eagle's medium), Fetal bovine serum, L-glutamine, non-essential amino acids and RPMI1640 were used for tissue culture.

Transfection

Transfection was carried out by the Effectene method (Promega) following the manufacturer's instruction. 1.0×10^5 cells were plated onto 60-mm plates and grow overnight. JCV genomic DNA with various was used for transfection. The total amount of transfected DNAs for the 35-mm plate was up to 1 mg. At day 14, 19, 25, 33, 39, 46, 50 and 57 after transfection, the cells were washed with phosphate-buffered saline and lysed with Passive Lysis Buffer. After a freeze/thaw cycle, samples were analyzed by Western blot using anti-JCV VP1 antiserum.

RESULTS AND DISCUSSION

The JCV noncoding transcriptional control region (TCR), containing the promoter and enhancer, can alter the cellular host range and may be responsible for switching JCV between states of lytic and latent infection. In addition, TCR is used to classify viral variants of JCV. It is suggested that different cell type susceptibility to JCV infection is due to its nuclear binding factors and TCR nucleotide sequences of JCV. To investigate the activity of transcriptional control region (TCR) of human JCV, TCRs of CY archetype, MAD-1 prototype, and ten Taiwan subtypes were cloned into JCV genomic DNA. The genomic DNAs with various TCR were transfected into human neuroblastoma cells by calcium phosphate precipitation method. Activity of TCR activity was determined by Western blot using anti-JCV VP1 antiserum. The Results indicated that TW-4 and TW-12 JCV genomes showed the strongest replication activity after transfection and determined by Western blot using anti-VP1 antiserum. TW5 and TW-11 did not show any replication activity. Virions with various TCR will be further isolated and used to infect human neuroblastoma cells for comparison of various TCR activities. These preliminary results demonstrated that promoter activity is correlated with different genotypes of JCV. Furthermore, activity of JCV TCRs may varied when expressed in different cell lines. These findings may provide important information for further studies in viral pathogenesis.

For the past few years we have been involved in the investigation of epidemiology and genotyping of human polyomaviruses in Taiwan. Four different strains, CY, Taiwan-1 (TW-1), Taiwan-2 (TW-2), and Taiwan-3 (TW-3) of JCV were found in pregnant individuals and autoimmune disease patients in Taiwan. The differences at the transcriptional control region between CY and Taiwan strains were that the Taiwan strains contain the deletions of pentanucleotide-A (GGGAA) and/or pentanucleotide-B (AAAGC). The role(s) of pentanucleotide-A and B in the physiology of the virus remains unknown. Tada and Khalili (25) found a brain-derived DNA binding protein LCP-1 (lytic control element-binding protein 1), capable of binding the lytic control element (LCE) (AGGGAAAGGGA) and regulating the early gene expression of JCV lytic cycle in glial cells. Furthermore, Kumar et al. (14) demonstrated that TGGAAAGCAGCCA was one of the nuclear factor 1 (NF-1) motifs important for glial cell-specific expression of JCV in differentiated embryonal carcinoma cells *in vivo*. More recently, Kumar et al. (15) showed that GGG regions in the LCE are required for glial cell-specific transcription. Moreover, Amemiya et al. (2) found that nuclear factor 1 of glial cells could bind to a specific site, site C (nucleotides 207-231), in the regulatory region of JCV, which contained AAAGC (PB-2) and GGGAA (PA-3), and it may involve gene regulation of JCV in glial cells. The nuclear factors which may be involved in the gene regulation of JCV replication will be further investigated.

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