行政院國家科學委員會專題研究計畫　成果報告

人類 JC 病毒基因調控之研究

計畫類別：個別型計畫
計畫編號：NSC92-2320-B-040-056-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：中山醫學大學醫學系微生物及免疫學科

計畫主持人：王梅林

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國　93 年 11 月 3 日

行政院國家科學委員會專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※
※ ※
※ **人類 JC 病毒基因調控之研究** ※
※ ※
※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：■個別型計畫

計畫編號：NSC 92-2320-B-040-056

執行期間：92 年 8 月 1 日至 93 年 7 月 31 日

主 持 人：王梅林

計畫參與人員：王梅林

執行單位：中山醫學大學醫學系微免科

中 華 民 國 93 年 10 月 24 日

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 a luciferase reporter system in human cells. The Results showed that activities of CY and Mad-1 were higher when transfected into human embryonic kidney（293）, colon adenocarcinoma（COLO320 HSR）, neuroblastoma（IMR-32）, breast adenocarcinoma（MCF-7）, and gastric carcinoma（SNU-1）. Subsequently, TCR activities of ten Taiwan subtypes of JCV were analyzed in these cell lines. Early promoter of TW-4 showed the strongest activity in each cell type when compared to other Taiwan subtypes. Human breast adenocarcinoma（MCF-7）provided the highest expression activity for the TCRs when compared to other cell lines. 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.

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 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.

MATERALS AND METHODS

Cell lines

pGL3-basic plasmid (Promega) was used as a reporter vector. TW-1~TW-12 JCV TCRs (Fig. 1) were cloned into pGL3-basic plasmid. Human neuroblastoma cell line (IMR32; CCRC-60014) was used for luciferase expression. Transfection kit (Promega), luciferase assay kit (Promega) and luciferase detection machine (Turner Designs 20/20) were purchased from the companies.

Human cell lines: embryonic kidney cell (CCRC-60019), skin cell (CCRC-60112), bladder cell (CCRC-60068), esophagus cell (CCRC-60166), colon cell (CCRC-60109), ovary cell (CCRC-60067), bone cell (CCRC-60225), brain glioblastoma multiforme cell (CCRC-60194), brain astrocytoma cell (CCRC-60193), lung adenocarcinoma cell (CRL-5865), lung squamous carcinoma cell (CRL-5826), T leukemia cell (CCRC-60248), intestine cell (CCRC-60022), brain neuroblastoma cell (CCRC-60014), skin xerderma pigmentosum cell (CCRC-60152), breast adenocarcinoma cell (CCRC-60221), B Burkit's lymphoma cell (CCRC-60116), and stomach carcinoma cell (CCRC-60210) were used for tissue specificity studies. DMEM (Dulbecco's modified Eagle's medium), Fetal bovine serum, L-glutamine, non-essential amino acids and RPMI1640 were used for tissue culture.

Transfection and Luciferase Assays

Transfection was carried out by the Effectene method (Promega) following the manufacturer's instruction. 1.0 x 10^5 cells were plated onto 60-mm plates and grow overnight. Vectors, including the reporter vectors, the internal *Renilla* luciferase control vector (pRLTK), and other expression vectors were co-transfected. The total amount of transfected DNAs for the 60-mm plate was up to 1 mg. All assays for firefly and *Renilla* luciferase activity were performed using one reaction tube sequentially (Promega). At 40 h post-transfection, the cells were washed with phosphate-buffered saline and lysed with Passive Lysis Buffer. After a freeze/thaw cycle, samples were mixed with Luciferase Assay Reagent II, and the firefly luminescence will be measured with a Luminometer (Turner Designs, CA). Next, samples were mixed with the Stop & Go reagent, and the *Renilla* luciferase activity was measured as an internal control. Finally, luciferase activity was calculated as follows: (firefly luciferase activity of the sample/*Renilla* luciferase activity of the sample) — (firefly luciferase activity of the background (control vector)/ *Renilla* luciferase activity of the background (control vector)). The results of each experiment were confirmed by three independent transfections.

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 a luciferase reporter plasmid. The plasmids were transfected into various human cell lines by calcium phosphate precipitation method. Activity of luciferase was used as an index of the corresponding TCR activity. Twenty two human cell lines were used for the assays.

The Results showed that activities of CY and Mad-1 were higher when transfected into human embryonic kidney（293）, colon adenocarcinoma（COLO320 HSR）, neuroblastoma（IMR-32）, breast adenocarcinoma（MCF-7）, and gastric carcinoma（SNU-1）. Subsequently, TCR activities of ten Taiwan subtypes of JCV were analyzed in these cell lines. Early promoter of TW-4 showed the strongest activity in each cell type when compared to other Taiwan subtypes. Human breast adenocarcinoma（MCF-7） provided the highest expression activity for the TCRs when compared to other cell lines. 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 JC V 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) (AGGGAAGGGA) 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.

REFERENCES

1. Agostini, H. T., Ryschkewitsch, C. F., and Stoner, G. L. (1996). Genotype profile of human polyomavirus JC excreted in urine of immunocompetent individuals. *J. Clin. Microbiol.* 34, 159–164.
2. Amemiya, K., Traub, R., Durham, L. and Major, E.O. (1992). Adjacent nuclear factor-1 and activator protein binding sites in the enhancer of the neurotropic JC virus. A common characteristic of many brain-specific genes. *J. Biol. Chem*. 267:14204-14211.
3. Ault, G. S., and Stoner, G. L. (1993). Human polyomavirus JC promoter/ enhancer rearrangement patterns from progressive multifocal leukoencephalopathy brain are unique derivatives of a single archetypal structure. *J. Gen. Virol.* 74, 1499–1507.
4. Chang, C. F., Gallia, G. L., Muralidharan, V., Chen, N. N., Zoltick, P., Johnson, E., and Khalili, K. (1996). Evidence that replication of human neutropic JC virus DNA in glial cells is regulated by the sequence-specific single-stranded DNA-binding protein Pur . *J Virol.* 70:4150–4156.
5. Ciappi, S., Azzi, A., De Santis, R., Leoncini, F., Sterrantino, G., Mazzotta, F., and Mecocci, L. (1999). Archetypal and rearranged sequences of human polyomavirus JC transcription control region in peripheral blood leukocytes and in cerebrospinal fluid. *J. Gen. Virol.* 80, 1017– 1023.
6. Daniel, A. M., Swenson, J. J., Mayreddy, R. P., Khalili, K., and Frisque, R. J. (1996). Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. *Virology* 216, 90–101.
7. Dorries, K., Vogel, E., Gunther, S., and Czub, S. (1994). Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. *Virology* 198, 59–70.
8. Feigenbaum, L., Khalili, K., Major, E. O., and Khoury, G. (1987). Regulation of the host range of human polyomavirus JCV. *Proc. Natl. Acad. Sci. USA* 84: 3695–3698.
9. Gronostajski, R. M. (2000). Roles of the NF1/CTF gene family in transcription and development. *Gene* 249:31–45.
10. Hara, K., Sugimoto, C., Kitamura, T., Aoki, N., Taguchi, F., and Yogo, Y. (1998). Archetype JC virus efficiently replicates in COS-7 cells, simian cells constitutively expressing simian virus 40 T antigen. *J. Virol.* 72:5335–5342.
11. Komagome, R., Sawa, H., Suzuki, T., Suzuki, Y., Tanaka, S., Atwood, W. J., and Nagashima, K. (2002). Oligosaccharides as recepteors for JC virus. *J. Virol*. 76:12992-13000.
12. Krynska, B., del Valle, L., Gordon, J., Otte, J., Croul, S., and Khalili, K. (2000). Identification of a novel p53 mutation in JCV-induced mouse medulloblastoma. *Virology* 274: 65–74.
13. Kunitake, T., Kitamura, T., Guo, J., Taguchi, F., Kawabe, K., and Yogo, Y. (1995). Parent-to-child transmission is relatively common in the spread of the human polyomavirus JC virus. *J. Clin. Microbiol.* 33, 1448–1451.
14. Kumar, K.U., Pater, A. and Pater, M.M.(1993). Human JC virus perfect palindromic nuclear factor 1-binding sequences important for glial cell-specific expression in differentiating embryonal carcinoma cells. *J. Virol*. 67:572-276.
15. Kumar, G., Srivastava, D.K. and Tefera, W. (1994). A 70-to 80-kDa glial cell protein interacts with the AGGGAAGGGA domain of the JC virus early promoter only in the presence of the neighboring cis DNA elements. *Virology*. 203:116-124.
16. Liu, C. K., Wei, G., and Atwood, W. J. (1998). Infection of glial cells by the human polyomavirus JC is mediated by an N-linked glycoprotein containing terminal (2-6)-linked sialic acids. *J. Virol.* 72: 4643–4649.

17. Markowitz, R. B., Eaton, B. A., Kubik, M. F., Latorra, D., McGregor, J. A., and Dynan, W. S. (1991). BK virus and JC virus shed during pregnancy have predominantly archetypal regulatory regions. *J. Virol.* 65, 4515– 4519.
18. Newman, J. T., and Frisque, R. J. (1999). Identification of JC virus variants in multiple tissues of pediatric and adult PML patients. *J. Med. Virol.* 58,79–86.
19. Pho, M. T., Ashok, A., and Atwood, W. J. (2000). JC virus enters human glial cells by clathrin-dependent receptor-mediated endocytosis. *J. Virol.* 74: 2288–2292.
20. Safak, M., Gallia, G. L., and Khalili, K. (1999). A 23-bp sequence element from human neurotropic JC virus is responsive to NF-岂B subunits. *Virology* 262: 178–189.
21. Safak, M., Gallia, G. L., Ansari, S. A., and Khalili, K. (1999). Physical and functional interaction between the Y-box binding protein YB-1 and human polyomavirus JC virus large T antigen. *J. Virol.* 73:10146–10157.
22. Shinohara, T., Nagashima, K., and Major, E. P. (1997). Propagation of the human polyomavirus, JCV, in human neuroblastoma cell lines. *Virology* 228: 269–277.
23. Sugimoto, C., Kitamura, T., Guo, J., Al-Ahdal, M. N., Shchelkunov, S. N., Otova, B., Ondrejka, P., Chollet, J. Y., El-Safi, S., Ettayebi, M., Gresenguet, G., Kocagoz, T., Chaiyarasamee, S., Thant, K. Z., Thein, S., Moe, K., Kobayashi, N., Taguchi, F., and Yogo, Y. (1997). Typing of urinary JC virus DNA offers a novel means of tracing human migrations. *Proc. Natl. Acad. Sci. USA* 94:9191–9196.
24. Sumner, C., Shinohara, T., Durham, L., Traub, R., Major, E. O. and Amemiya, K. (1996). Expression of multiple classes of the nuclear factor-1 family in the developing human brain: differential expression of two classes of NFI genes. *J. Neurovirol*. 2:87-100
25. Tada, H., and Khalili, K. (1992). A novel sequence-specific DNA-binding protein, LCP-1, interacts with single-stranded DNA and differentially regulated early gene expression of the human neurotropic JC virus. *J. Virol*. 66:6885-6892.
26. Vacante, D. A., Traub, R., and Major, E. O. (1989). Extension of JC virus host range to monkey cells by insertion of a simian virus 40 enhancer into the JC virus regulatory region. *Virology* 170: 353–361.
27Weber, T., and Major, E. O. (1997). Progressive multifocal leukoencephalopathy: Molecular biology, pathogenesis and clinical impact. *Intervirology* 40:98–111.
28. White, F., Ishaq, M., Stoner, G. L., and Frisque, R. J. (1992). JC virus DNA is present in many human brain samples from patients without progressive multifocal leukoencephalopathy. *J. Virol.* 66:5726– 5734.
29. Winklhofer, K. F., Albrecht, I., Wegner, M., and Heilbronn, R. (2000). Human cytomegalovirus immediate-early gene 2 expression leads to JCV replication in nonpermissive cells via transcriptional activation of JCV T antigen. *Virology* 275: 323–334.
30. Yogo, Y., Guo, J., Iida, T., Satoh, K., Taguchi, F., Takahashi, H., Hall, W. W., and Nagashima, K. (1994). Occurrence of multiple JC virus variants with distinctive regulatory sequences in the brain of a single patient with progressive multifocal leukoencephalopathy. *Virus Genes* 8:99–105.
31. Yogo, Y., Iida, T., Taguchi, F., Kitamura, T., and Aso, Y. (1991). Typing of human polyomavirus JC virus on the basis of restriction fragment length polymorphisms. *J. Clin. Microbiol.* 29, 2130–2138.
32. Yogo, Y., Kitamura, T., Sugimoto, C., Ueki, T., Aso, Y., Hara, K., and Taguchi, F. (1990). Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. *J. Virol.* 64, 3139–3143.
33. Zambrano, A., Kalantari, M., Simoneau, A., Jensen, J.L., and Villarreal, L.P. (2002). Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. *Prostate*. 53:263-276.