行政院國家科學委員會專題研究計畫 成果報告

大腸癌之基因治療，利用人類多瘤性病毒，JC 病毒之類病毒顆粒作為基因輸送載體

研究成果報告(精簡版)

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行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

（計畫名稱）

Gene therapy of colorectal cancer using the human polyomavirus, JC virus, virus-like particle as gene delivery vector

計畫類別：■ 個別型計畫　□ 整合型計畫

計畫編號：NSC 95-2320-B-040 -006

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計畫主持人：王梅林

共同主持人：

計畫參與人員：

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The ultimate goal of developing gene transfer vectors is to establish a effective and safe treatment for human diseases which include genetic disorders, cancer, cardiovascular disease and infectious diseases. Development of gene transfer vectors has been intensively investigated for years. Current used methods for gene delivery include non-viral and viral methods. Non-viral methods employ liposome, direct DNA injection, calcium phosphate transfection, particle bombardment (gene gun) and electroporation to transfer genes of interest into cells. Viral vectors include retroviruses, adenovirus, adeno-associated virus, pox virus and herpes simplex virus . Few studies have focused on development of a gene transfer vector using human polyomavirus, partly due to the difficulty in propagating the virus in cell culture and subsequent limited availability of the viral particles. In addition, mechanism(s) of viral assembly and DNA encapsidation is not well documented. Based upon the knowledge in regard to assembly and gene delivery of polyomavirus, it is feasible to use human polyomavirus, JCV, as a gene transfer vector.

The human polyomavirus JC virus is widespread in human population. Primary infection occurs in childhood and persists latently within the renal system. Pregnancy or immunosuppressing conditions result in reactivation of the virus and cause viruria . Our previous results also showed that 13.3% of immunocompetent individuals, 26.0% of pregnant women and 37.5% of autoimmune disease patients are JCV positive. All of the immunocompetent individuals are BKV negative, but 3.9% of pregnant women and 6.2% of autoimmune disease patients are BKV positive. These results suggest that the incidence of urinary excretion of human polyomaviruses in immunosuppressed individuals is higher than that of immunocompetent individuals and the prevalence of JCV appears to be higher than that of BKV in Taiwan. Four different genotypes, CY, Taiwan-1 (TW-1), Taiwan-2 (TW-2), and Taiwan-3 (TW-3) of JC virus (JCV) and two BK virus (BKV) variants, Taichung-1 (TC-1) and Taichung-2 (TC-2) were found in pregnant individuals and autoimmune disease patients in Taiwan. Recently, some other new genotypes of JCV and BKV were also identified in patients with renal transplant. The genotypes were designated TW-4 through TW-12 with GenBank accession numbers AF218436 through AF218444 for JCV and TC-3 through TC-7 with accession numbers AF164514 and AF218445 through AF218448 for BKV. These genotypes contained tandem repeats and/or deletions within the regulatory regions might be involved in tubulointerstitial nephritis.

JC virus is also active in glial cells of brain, causing a fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). The incidence of PML has increased dramatically in recent years as a result of the AIDS epidemic. It has been shown that tat protein of HIV can strongly activate JCV promoter for late gene transcription. However, it is still possible that the reactivation of JC virus in brain tissue of PML patients is caused by immunodeficiency conditions accompanied with HIV infection. In addition, JCV was able to develop glioblastomas, medulloblastomas and other unclassified primitive tumors in the inoculated hamsters. These transformed hamster cells demonstrated an integrated JCV genome that expressed the viral T antigen that could bind the

cellular p53 protein and retinoblastoma protein. Recent reports including our unpublished findings showed that JCV may cause various human brain tumors including oligoastrocytoma, astrocytoma, oligoastrocytoma, gliosarcoma, ependymoma, and subependymoma.

Reports of the presence of JCV in the urine and kidneys of normal individual suggest that a primary infection might involve an oral or respiratory route of transmission. During primary infection, there is viremia and the virus is transported to the kidney, where it persists indefinitely. It is not clear the relationship between JCV infection in the kidney and the brain. It has been found that JCV associated with peripheral blood lymphocytes in most of PML patients. Therefore, it have been hypothesized that B-lymphocyte is a possible circulating carrier of JC virus to the brain. Although JCV is frequently found in kidney, B lymphocytes and brain tissues, the virus has also been detected in spleen, lung, lymph node, and cardiac muscle of PML patients and in hematopoietic progenitor cells, primary B lymphocytes and tonsil tissue of immunocompetent individuals.

Human JC virus is a member of polyomavirus. The group of polyomavirus includes simian virus 40 (SV40), murine polyoma virus and human BK virus. The structure and genetic organization of JC virus are similar to other polyomaviruses. The JC virus genome is a closed circular double-stranded DNA molecule of 5.1 kb and encodes six proteins, two nonstructural tumor antigens, agnoprotein and three structural capsid proteins. The virus is composed of an icosahedral capsid made up of 72 pentamers of the major capsid protein VP1, the two minor proteins VP2 and VP3, circular supercoiled DNA, and host-contributed histones. The genome replicates bidirectionally starting from a unique origin. The early and late transcriptions begin near the replication origin and proceed in opposite directions on separate DNA strands. The early region encodes for the large T and small t antigens. In the lytic cycle the structural proteins VP1, VP2 and VP3 are synthesized from the late mRNAs. The three late proteins, VP1, VP2 and VP3, comprise the structural units responsible for maintaining the structural integrity of the JC virus; and they are also essential as the virus attachment protein (VAP) sites for infection of host cells, agglutination of erythrocytes and are essential in DNA packaging and virus assembly. The structure of simian virus 40 has been crystallographically determined. The shell of SV40 is composed of 360 copies of VP1, organized into 72 pentamers. The pentamers are situated at the points of a T=7d icosahedral surface lattice. There are twelve 5-coordinated pentamers and sixty 6-coordinated pentamers. Five VP1 subunits are intimately interlocked in a pentamer. Our results suggest that the twelve amino-terminal and sixteen carboxy-terminal amino acids of JCV VP1 are dispensable for the formation of irus-like particles, and further truncation at either end of VP1 leads to the loss of their interlocks in a pentameric structure. Disulfide bonds and calcium ions are essential for keeping the integrity of JCV capsid. Disulfide bonds were found in the JCV capsid and caused dimeric and trimeric VP1 linkages. In addition, disulfide bonds were further demonstrated to play an important role in maintaining the integrity of the JCV capsid structure by protecting calcium ions from chelation. The divalent cation calcium ion is an integral part of the JC virus and play a major role in

stabilizing the intact virion structure, and likely involved in the processes of viral uncoating and assembly. The recent results suggest that Asp239 and Asp338 of JCV VP1 were involved in calcium binding and then cause the inter-pentameric molecule association. The large BC, DE and HI of loops from each VP1 subunit interlock to form the top surface of the pentamer. These loops exposed on surface of the JCV VP1 were demonstrated to be important interacting domains for hemagglutination and antigenecity. Therefore, alterations of these surface domains may also change the tissue specificity of the virus.

The capability of polyomavirus capsids to serve as a gene transfer vector for gene therapy purposes has been investigated. In 1970, Osterman et al. have demonstrated that murine polyoma pseudovirions which encapsidates host DNA fragments are able to adsorb to mouse embryo cells and cause uncoating within the cells. In 1974, Kashmiri et al. further demonstrated that murine polyoma pseudovirions are able to delivery pseudoviral DNA (host DNA fragments) into mouse embryo and kidney cells. These findings suggest that polyoma pseudovirions could be as potential agents for mammalian gene transfer or transduction. Thereafter, in vitro DNA packaging by using in vivo propagated empty capsid of murine polyomavirus was further investigated. The DNA packaged by polyoma empty capsid was resistant to pancreatic DNase digestion. In addition, polyoma empty capsid was able to deliver a transforming gene into rat cells and then consequently cause transformation. Gene transfer by the empty capsid was 50 to 150 times more efficient than by the calcium phosphate precipitation method. More recently, Forstova et al. demonstrated that murine polyomavirus VP1 pseudocapsids generated from a recombinant baculovirus are able to transfer heterologous DNA efficiently into mammalian cells. Simian SV-40 pseudovirions self-assembled in insect cells have also been shown to be able to introduce foreign genes into mammalian cells. Our recent study demonstrated that human JCV VP1 self-assembled into virus-like particle (VLP) when expressed in insect cells, E. coli and yeast). The VLP was capable of packaging exogenous DNA in vitro and delivering the DNA into human kidney cells for expression. Furthermore, our current results also showed that JC VLP was able to delivery antisense oligodeoxynucleotide (ODN) against SV40 LT into COS-7 cells and inhibit the oncoprotein, LT, expression. In addition, JCV VLP infected human brain tumor cells including neuroblatoma and glioblastoma with high efficeicy. These findings will be important for further applications of human gene delivery with JCV VLP instead of using murine polyoma or simian SV40 capsids.

JC virus is a well-characterized etiological agent for the neurological disorder, PML, due to its lytic infection of the myelin-producing oligodendrocytes. In addition, it may also cause various human brain tumors. These evidences support that it is possible to use JCV VLP for transducing a gene of interest into its nature host for therapeutical purposes including brain tumors and other neurological disorders such as Parkinson’s disease and Huntington’s disease. Besides human brain, JCV was also found in kidney, spleen, lung, lymph node, cardiac muscle, hematopoietic progenitor cells, primary B lymphocytes and tonsil tissue. Therefore, investigation of tissue tropism of JCV appears to be essential to clarify tissue specificity when employing JCV VLP for gene targeting. t is

difficult to propagate human JC virus in cell culture due to a very restricted host cell range in vitro resulting in limited information in respect to the biological functions of the virus. Although JCV grows well in cultures of primary human fetal glial (PHFG) tissue, there are several difficulties of propagating JCV in the culture, such as that PHFG is difficult to obtain ; lytic activity of JCV in the cells is low, growth cycle prolonged, and CPE difficult to recognize; cells must be maintained for months before virus is harvested; and once established in culture, the cells rapidly lose the ability to support JCV replication. Therefore, it appears to be not possible to obtain enough JCV capsid particles from in vivo tissue culture for gene delivery investigation.

Recently, we have cloned and expressed the major capsid protein VP1 of JC virus in insect cells, E. coli and yeast. The recombinant VP1 protein was able to self-assemble into a capsid-like structure in both eukaryotic and prokaryotic cells. We have also demonstrated that the JCV VP1 protein has DNA binding and DNA packaging activities. The in vitro packaged plasmid DNA could be delivered and expressed in human kidney cells by JCV VP1 pseudocapsids indicating that the JCV vector can introduce the gene interest without viral genetic information, in contrast to the situation with adenovirus and retroviral vectors, into the recipient host. JC virus is able to productively infect human nondividing glial cells and is presumably capable of nonproductively infecting many human tissues. Therefore, JCV pseudocapsid may also be able to transduce the gene of interest into many human cells. Taking advantage of its ability to self-assemble, we are able to purify JCV VP1 capsid-like particles with abundance and ease for further gene delivery investigation.