行政院國家科學委員會專題研究計畫成果報告

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計畫類別：■個別型計畫

計畫編號：NSC 89-2320-B-040-006

執行期間：88 年 8 月 1 日至 89 年 7 月 31 日

主 持 人：張德卿

執 行 單 位：中山醫學院醫學系微免科

中　華　民　國　89　年　10　月　24　日

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Abstract

A renal allograft transplant patient with high serum creatinine presented one of clinical symptoms of rejection. Sections of renal biopsy tissue showed mononuclear leukocyte infiltration in the tubulointerstitium and nuclear enlargement with inclusions in the tubular epithelium. The morphological characteristics resembled polyomavirus-induced interstitial nephritis. Electron microscopy of the nuclear inclusions showed paracrystalline arrays of naked viral particles with a diameter of 45 nm. Molecular approaches revealed that a new variant of BKV with rearrangement at the regulatory region was involved in the nephritis. The BKV regulatory region contained a tandem repeat from the P-block to the Q-block causing duplication of several important transcriptional elements or transcriptional factor binding motifs. This is the first report to show a naturally occurring BKV variant with regulatory region rearrangement associated with tubulointerstitial nephritis.

Keywords: BKV; renal transplant; interstitial nephritis

Introduction

The human polyomaviruses, JC virus (JCV) and BK virus (BKV), are endemic in the human population [14]. The antibody prevalence to human polyomaviruses reaches nearly 80% by adult age [6]. Infection with polyomaviruses in healthy individuals is rarely associated with disease, although the viruses may lay latent indefinitely in the kidney [15]. Human polyomaviruses more frequently cause diseases in patients with immunosuppression, such as AIDS patients [9] and renal transplant recipients [3].

Renal transplantation is a better treatment for most patients with end-stage renal disease than dialysis therapies. Infectious complications, resulting in significant morbidity and mortality, remain a major problem in renal transplant patients. The issue has become more important in recent years, as several new immunosuppressant agents have been introduced for treating organ transplant patients. Most previous studies on renal pathogenesis of BKV were based on histological approaches [2]. Therefore, the possible correlation between the BKV variant and renal pathogenesis has not been clearly illustrated. In this study, a renal transplant recipient was diagnosed as having tubulointerstitial nephritis. A new variant of BKV with a rearranged regulatory region was identified, and it was associated with the nephritis as demonstrated by histological and molecular approaches.

MATERIALS AND METHODS

Renal Allograft Recipient

The patient was a 30-year-old male with an end-stage renal disease. After hemodialysis treatment for 7 months, he underwent cadaveric renal transplantation in mainland China. Afterwards, the patient returned to Taiwan and received medical care at the Division of Nephrology,

Taichung Veterans General Hospital.

Histology and Urine Cytology

Renal tissues were collected by needle biopsy during clinical manifestation with suspicions of acute rejection. The histological evaluation was carried out according to standard protocols by light microscopy (hematoxylin and eosin, periodic acid-Schiff, Masson's trichrome, and methenamine silver stains) accompanied by immunofluorescence microscopy and electron microscopy.

Immunohistochemistry

The biopsy specimens were fixed in 10% neutral phosphate-buffered formalin, and were embedded in paraffin. The thin-sectioned specimens were deparaffinized and rehydrated. The target retrieval procedure involves the immersion of tissue sections into a target retrieval solution prior to immunostaining.

Polymerase Chain Reaction

The biopsied tissue was incubated with 20 *u*l of lysis buffer containing proteinase K (10 mM Tris-HCl, 1 mM EDTA, pH 8.0 and 50 *u*g/ml proteinase K) at 50 °C for overnight. The mixture was centrifuged at 10,000 rpm for 3 min. Two microliters of the supernatant was used for polymerase chain reaction (PCR). The detailed protocols for PCR were described previously [4].

Electrophoresis

Five microliters of the PCR reaction mixture of each sample was loaded onto a 1.5% agarose (IBI Biotechnologies, CN, USA) gel and subjected to electrophoresis in TBE buffer (90 mM Tris-HCl, pH 8.3, 90 mM sodium borate, 2.5 mM EDTA). The agarose gel was stained with ethidium bromide to visualize the band under UV light.

Southern Blot

Ten microliters of the PCR reaction mixture was electrophoresed in a 1.5% agarose gel in TAE buffer (40 mM Tri-acetate, pH 8.5, 2 mM EDTA). The DNA fragments in the agarose gel were transferred onto a nylon membrane using semi-dry Horizblot. The detailed protocols for Southern blot were described previously [4].

DNA Sequencing

The DNA fragment in the low melting agarose gel was purified with a Magic PCR Minipreps kit (Promega). The purified DNA fragment was directly sequenced by the *f*mol DNA sequencing kit purchased from Promega. The detailed protocols for DNA sequencing were provided by the company.

Results

Clinical Manifestation after Renal Transplantation

The patient developed oliguria a week after transplantation. Anti-lymphocyte globulin (ALG) was administrated for 2 weeks under the impression of acute rejection. His renal function improved steadily with a serum creatinine level of 2.0 mg/dl when he was on triple therapy with cyclosporine (CyA), prednisolone and mycophenolate mofetil (MMF). A second episode of acute rejection occurred 1 month later, which prompted a change from CyA to FK506. This was complicated by a herpes zoster infection over the left T12-L1 dermatome soon after the switch. Within the following months, many episodes of acute rejection occurred. The patient received three courses of recycle prednisolone therapy, two courses of methylprednisolone pulse therapy, and two courses of ALG therapy.

Identification of Polyomavirus Infection

A renal graft biopsy was performed. Light microscopy of the biopsy specimen revealed tubulointerstitial mononuclear leukocyte infiltrates surrounding the tubular epithelium with nuclear enlargement and intranuclear inclusions, which was consistent with polyomavirus infection-induced interstitial nephritis. No cytoplasmic

inclusion bodies were present. Immunofluorescence microscopy did not show depositions of immunoglobulins or complement factors directed against a routine panel of antibodies. Furthermore, electron microscopy showed that the nuclear inclusion bodies consisted of paracrystalline arrays of naked, round, electron-dense viral particles measuring 45 nm in diameter. The morphology of these virions was a characteristic of polyomavirus. Cytomegalovirus and herpes simplex virus were not detected in the biopsy tissue by immunohistological staining. Urine cytology disclosed intranuclear inclusion cells, so-called "decoy cells", with an enlarged nucleus and clumping of the chromatin around the nuclear border. Large intranuclear, opaque basophilic inclusions of decoy cells are morphological characteristics of BKV activation and replication.

Identification of the BKV variant

To further distinguish BKV and identify the viral genotype involved in the pathogenesis, the biopsy tissue was examined by PCR, Southern blot, and DNA sequencing [4]. The PCR product containing the viral regulatory region showed a DNA fragment of approximately 380 base pairs (bp) in agarose gel electrophoresis. The DNA fragment was further confirmed as a sequence of human polyomavirus regulatory region by Southern blot. DNA sequencing revealed that a new variant of BKV designated as Taichung-3 (TC-3) was involved in the tubulointerstitial nephritis. The regulatory region of TC-3 contained a 54-bp nucleotide repeat from nucleotides 49 to 102 and 7 nucleotide alterations, 46A→T, 59T→C, 137C→A, 193C→T, 221A→G, 270G→T, and 279G→T (Fig. 4), when compared with the regulatory region of WW BKV variant [Rubinstein et al., 1987]. The GenBank accession number for the TC-3 sequences is AF164514.

Discussion

Most patients with renal allograft transplantation need to receive immunosuppressive agents for a long period of time to reduce rejection. BK virus infection or reactivation has been reported to be associated with ureteral stenosis [5], renal dysfunction [10], acute tubulointerstitial nephritis, or coexisting allograft rejection [11] in renal transplant recipients. In addition, BKV was also associated with viral pancreatitis, hemorrhagic cystitis or transient hepatic dysfunction in bone marrow recipients [1], while the patients were receiving immunosuppressant agents. Although BKV infection is recognized to be involved in interstitial nephritis in renal transplant patients, it is still not clear why only a small number of immunosuppressed patients with BKV infection develop renal diseases [12]. Prevalence of human polyomavirus infection in renal transplant patients at our hospital is also undergoing investigation. Currently, only one patient out of about 300 was diagnosed with interstitial nephritis and the patient was infected by a regulatory region rearranged BKV variant. Whether the rearrangement of the regulatory region of BKV plays an important role in pathogenesis needs to be further investigated.

The TC-3 variant found in the interstitial nephritis tissue is a BKV variant which occurs naturally. The anatomy of the regulatory region of the TC-3 variant contains a tandem repeat from P-block 21 to Q-block 6 according to the P, Q, R nomenclature proposed by Markowitz and Dynan [7]. The tandem repeat duplicates several important transcriptional elements or transcriptional factor binding motifs, such as NF-1, the *c-mos* upstream enhancer, phorbol-inducible element in plasminogen activator inhibitor type 2 promoter, PEA3, CMV *ie*-1 promoter, LF-A1, and SP1 [7]. Whether the duplication of these transcriptional elements at TC-3 BKV increases reactivity or virulence will be further investigated.

In renal allografts, polyomavirus induces symptoms characterized by acute tubulointerstitial nephritis, simulating acute cellular rejection, which makes the decision-making for an immunosuppression regimen more difficult. It has been reported that BKV excretion was detected after an episode of rejection [8]. It has been suggested that BKV is reactivated after the administration of immunosuppressive treatments [3]. Some polyomavirus-infected renal grafts with tubulointerstitial nephritis did not improve after receiving immunosuppressive agents [10]. In contrast, attempts to lower the immunosuppression stabilized many renal allografts as reported previously [8]. In the current case, although FK 506 was replaced immediately by CyA to reduce immunosuppression after the second biopsy, the renal graft still lost its function. It is possible that the early detection of polyomavirus infection and the subsequent early relief of immunosuppression may stabilize graft function [8].

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