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２００３年度日中医学協会共同研究等助成事業報告書

－在留中国人研究者研究助成－

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財団法人 日中医学協会
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１．研究テーマ

腎移植患者と腎結石患者におけるBKウイルス亜型の感染率と臓器内分布

２．本年度の研究業績

(1) 学会・研究会等における発表 ☑有 ・ 無 （学会名・演題）

第９２回日本病理学会総会

BK virus infection in renal transplant recipients

(2) 学会誌等に発表した論文 有 ・ ☑無 （雑誌名・論文名）

3．今後の研究計画

To detect BK virus in carcinomas because BK virus is a potential oncogenic tumor virus.

To detect JC virus in urine samples from the renal transplant recipients and urinary lithiasis patients, and then analyze the sequence of JC virus genome of positive cases.

To collect urine samples of the renal transplant recipients from China and detect BK virus and JC virus, and compare whether there are differences between in China and in Japan.

4．指導責任者の意見

韓先生は日本に来てから間もない期間に片言ながら日本語が話せるようになり、大学の雰囲気にも慣れてきています。BK ウイルスの研究のきっかけは移植後の患者で尿細胞診の中に封入体が見つかったことが契機でした。形態的にポリオーマウイルスと考えられ、BK ウイルスと同定されました。韓先生はこの症例をきっかけとして、他の移植例やコントロールとして結石症患者の尿沈さや組織を用いて BK ウイルスの検出を試みました。さらに陽性例についてはウイルスの型を RFLP 法や配列を直接決定して詳細な検討をおこないました。短期間の割には着実な成果を挙げられ、現在別のポリオーマウイルスである JC ウイルスにも研究が広がっています。さらには論文の抄読会や病理診断の検討にも加わって精力的に仕事をこなしています。

指導責任者氏名 三浦克敏 ㊞

5．研 究 報 告 書

別紙「研究報告書の作成について」に倣い、指定の用紙で作成して下さい。

研究発表または研究状況を記録した写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

※発表に当っては、*日中医学協会助成金による*旨を明記して下さい。

－日中医学協会助成事業－

腎移植患者と腎結石患者におけるBKウイルス亜型の感染率と臓器内分布—

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Abstract

We collected urine samples of renal transplant recipients and urinary lithiasis patients and tissue specimens from autopsy individuals received renal transplantation. 327 base pairs of VP1 region and 149 base pairs of large T antigen were amplified by PCR and nested-PCR, respectively. The infective rates of BK virus are high compared to normal population. BK virus subtype is predominantly type Ⅰ. Immunohistochemistry can identify BK virus location combining with histopathologic changes, but its specificity is questionable. PCR is a sensitive and rapid method for detecting BK virus after renal transplantation before the histopathological demonstration and provides a useful opinion for monitoring immunosuppressive therapy.

Key words　BK virus　renal transplantation　urinary lithiasis

Purpose

Primary BK virus infection occurs during childhood. After the primary infection, the virus remains latent in blood cells and in the urinary tract, and can be reactiviated in some conditions such as immunocomprised state, pregnancy, and diabetes. In recent studies, BK virus and JC virus have been implicated as a cause of interstitial nephritis in renal transplant recipients. Immunosuppressive treatment with tacrolimus and mycophenylate mofetil appears to increase the risk of viral reactiviation in renal transplant recipients. Four BK virus genotypes with characteristic amino acid at VP1 gene product are identified. In our research, we revealed BK virus infection in renal transplant recipients and urinary lithiasis patients, resolved BK virus subtype and determine DNA sequence of VP1 region, and showed localization of BK virus in tissues by immunohistochemistry and in situ hybridization.

Materials and methods

1. Collecting urine samples and autopsy paraffin sections.
   241 and 113 urine samples from renal transplant recipients and urinary lithiasis patients. 12 kidney sections, 12 lung sections, and 3 brain sectionss from 13 autopsy individuals.
2. Purification of DNA from these samples.
3. Checking DNA status by beta-globin amplification using PCR.
4. Amplify BK virus genome by PCR.
   For beta-globin positive DNA, 327 base pairs from BK virus VP1 region was amplified using urine DNA. 149 base pairs from BK virus large T region was amplified using autopsy specimens by nested-PCR.
5. Determining BK virus subtype from VP1 genome by restriction endonucleases digestion using XmnI, RsaI, AvaII, and AluI.

6. DNA sequencing.

PCR products were seperated by 3% agarose gel electrophoresis. Bands corresponding to BK virus viral capsid protein VP1 products were cut and dissolved using Geneclean kit. Sequencing reactions were carried out by sequencing kit.

7. Immunhistochemical detection of BK virus by anti-SV40 T antibody.

8.Detection of BK virus genome by in situ hybridization with BK virus whole probe.

Results

1.twelve of 71 β-globin positive renal transplant cases were positive for BK virus VP1 region, in which 11 cases were type I and 1 case is type Ⅳ. Two of 20 β-globin positive urinary lithiasis cases expressed BKV VP1 region, which were type Ⅰ. In 13 autopsy cases of renal transplantation, 7 /13 were positive for BK virus, 7/12 in the kidney, 5/12 is involved in the lung, and 1/3 in the brain. (These results were shown in Table 1,2,3 and Figure 1,2,3).

2.The sequences of one renal transplant recipient are consistent with BK virus.

3.Immunohistochemistry showed that BK virus was located in the nulei of tubular epithelium and interstitial cells, but non- specific staining is conspicuous. True positive cells showed enlarged nuclei with ground-glass appearance. (Figure 4)

4.BK virus cannot be detected in situ hybridization

Conclusion

1. Rate of BK virus infection in renal transplantation recipients (16.90%) and in urinary lithiasis (10%) is higher than that of normal population (0.3%). For autopsy cases, the infective rate of BK virus (53.8%) is more than half cases.
2. The BK virus subtype in renal transplant recipients was predominantly type Ⅰ.
3. Immunohistochemistry is a useful method for detecting BK virus location, but its specificity is questionable. Evaluation with histological changes is necessary.

Discussion

BK virus as a human polyomavirus has been associated with urinary tract stenosis in renal transplant patients and hemorrhage cystitis in bone marrow transplant patients, virus isolation and Southern hybridization analysis demonstrate the kidney is the main site of BK virus latency in healthy population. however PCR analysis indicates that BK virus establishes latent infection in multiple organs. Latent BK virus infection of the renal epithelium is reactivated by immunosuppressive state, leading to viruria. Asymptomatic viruria with BK virus occurs in 0.3% of non-immunosuppressed patients and 10-45% of renal transplant recipients. Our results are consistent with these results. The high infective rates of autopsy cases after renal tranplantation demonstrate that BK virus may cause graft failure.

Literature

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注：本研究は，２００３年４月２４日「「第９２回日本病理学会総会」」にて示説発表しました。

作成日：2004年３月 ５日

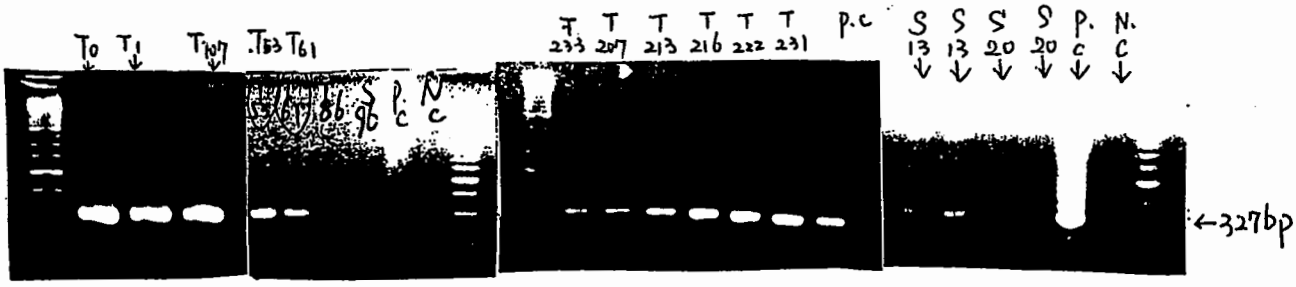
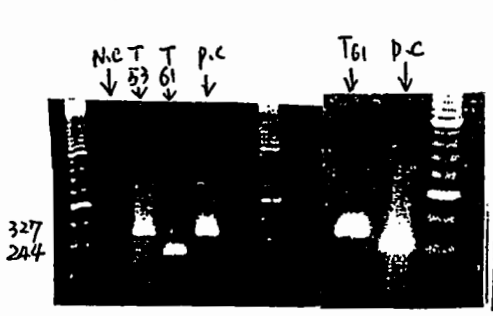


Figure 1



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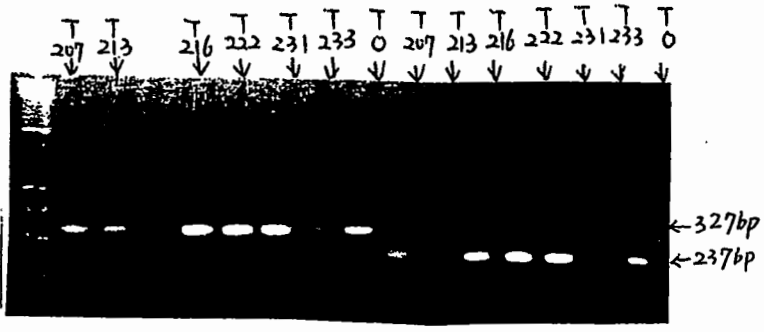

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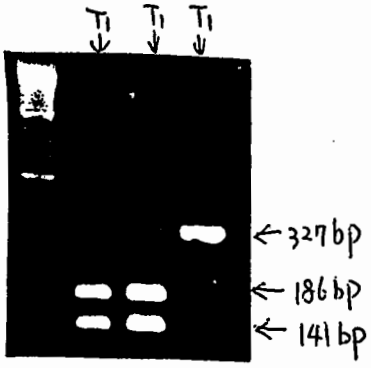

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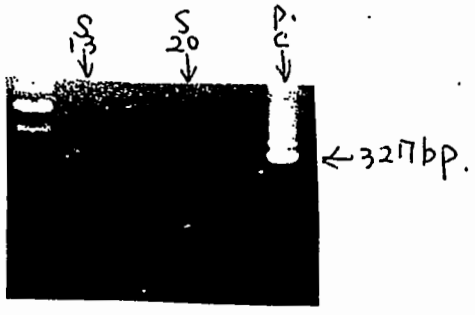

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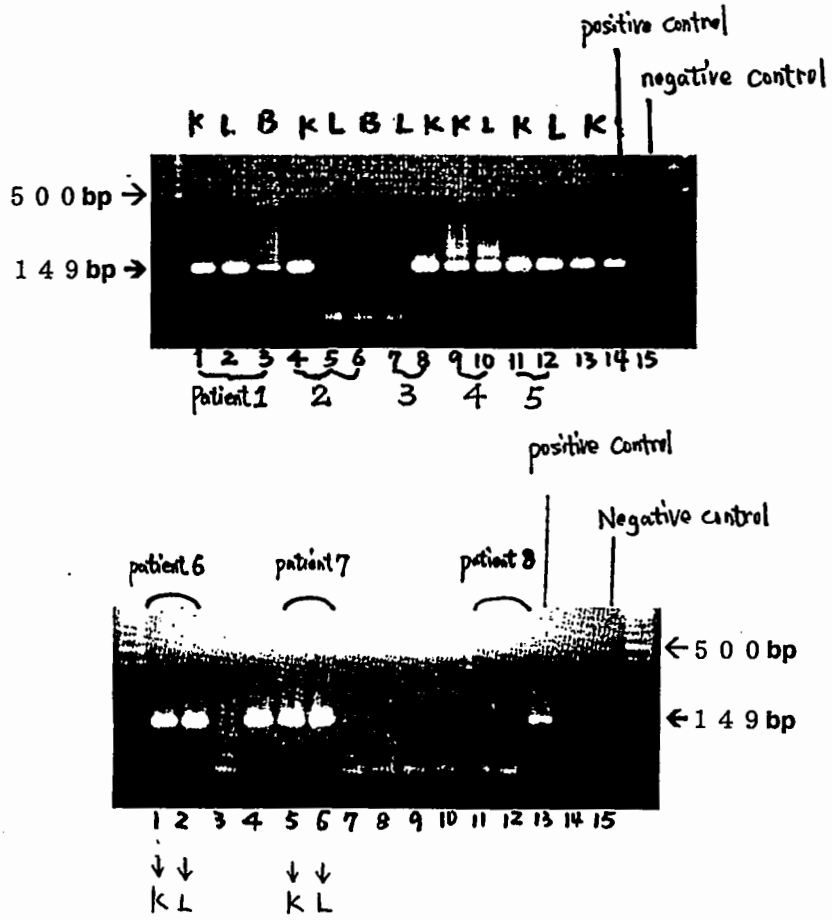

Figure 3

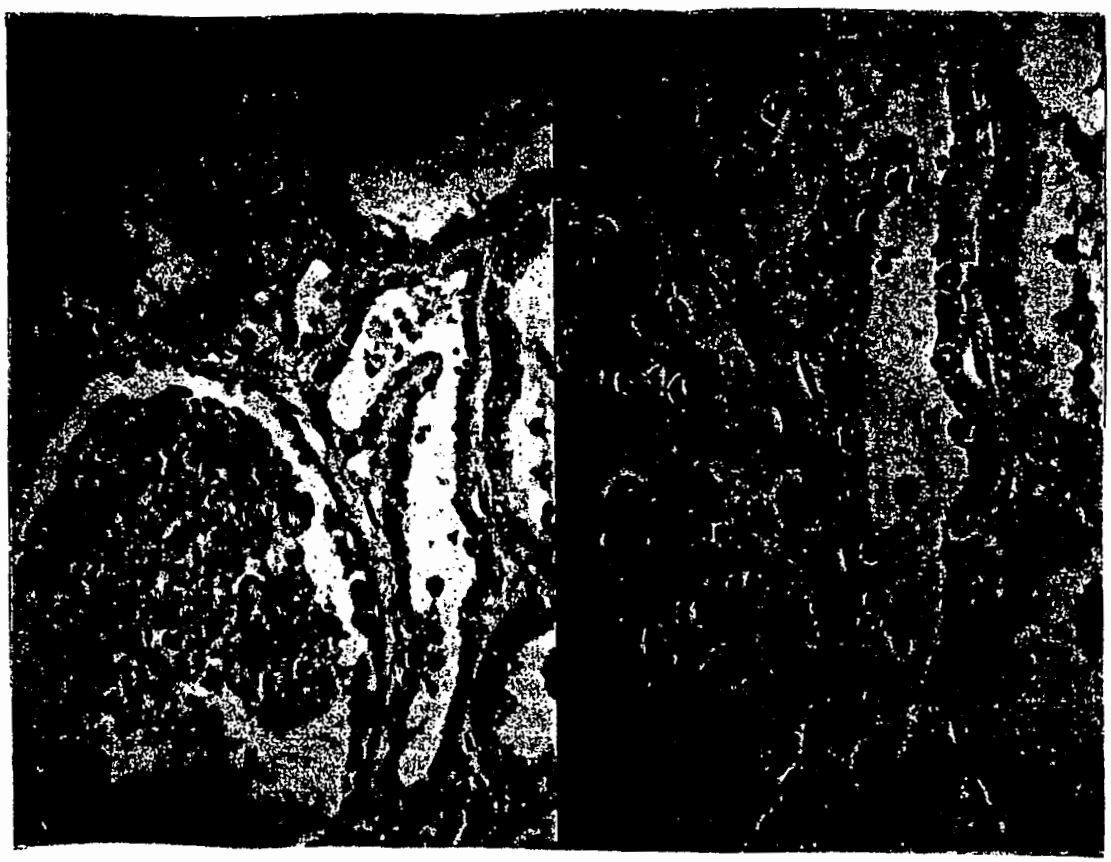

Figure 4

Figure legend

Figure 1. The electrophoresis results of PCR products in renal transplant recipients and renal lithiasis patients. 327 base pairs of BK virus VP1 region was amplified in BK virus positive cases. The numbers on the images stand for the number of patients. T: renal transplant recipients. S: urinary lithiasis. P.C: positive control. N.C: negative control

Figure 2. The electophoresis results show restriction endonucleases digestion of PCR products for subtyping. Left image of 2A, left half of 2B and 2D. Digest VPI region of BK virus with XmnI showed that the BK virus subtype I was uncut , and the BK virus subtype Ⅳ of T61 patient was cleaved to develop 244 and 83 base pairs . The right image of 2A shows that BK virus subtype Ⅳ of T61 patient was uncut with RsaI. The right half of 2B showed that the 327 base pairs bands were cleaved with AvaII to develop 237 and 90 base pairs. 2C. Digest 327 base pairs of PCR products with AluI show that the BK virus subtype I was cleaved to form 186 base pairs and 141 base pairs.
The numbers on the images stand for the number of patients. T: renal transplant recipients. S: urinary lithiasis. P.C: positive control. N.C: negative control.

Figure3. The electrophoresis results of nested-PCR products in autopsy cases of renal transplantation by BK virus large T antigen. 149 base pairs were amplified in positive cases. The symbols on and under the images stand for names of organ. K: kidney. L: lung. B: brain.

Figure 4. Immunohistochemistry showed that some nuclei of tubular epithelial cells are positive for SV-40 large T antigen expressed by BK virus.

Table 1. BK virus positive cases of urine

number / disease	Total numbers of urine samples	BK virus positive cases/ β-globin positive cases (%)	BK virus posttive case I II III IV
renal transplantation	243	12/71 (16.9)	11 1
renal lithiasis	113	2/20 (10)	2

Table 2. BK virus subtypes of positive urine samples

Patient number	BK virus subtype
T0	I
T1	I
T53	I
T61	IV
T107	I
T149	I
T207	I
T213	I
T216	I
T222	I
T231	I
T233	I
S13	I
S20	I

T: transplantation S:Urinary lithiasis

Table 3. BK virus positive cases in renal transplant autopsy on paraffin blocks with PCR amplification

Organs detected	Positive percentage of BK virus
Kidney	7/12
Lung	5/12
Brain	1/3