Detection of SV40 in Patients with Kidney Disease

Jeffrey Kopp, MD
Kidney Disease Section
NIDDK, NIH
Nephrotic syndrome

- Normal protein excretion: <150 mg/d
- Nephrotic syndrome: >3.5 g/d
  Often with edema, hypoalbuminemia, hypercholesterolemia
- Systemic nephrosis: diabetes, lupus
- Primary nephrosis
Focal Segmental Glomerulosclerosis

- Idiopathic FSGS, described 1957, now the leading cause of 1° NS
- HIV-associated FSGS
- Collapsing FSGS, new disease ~1980
- Hyperfiltration injury
- Genetic/familial
Why suspect SV40 in FSGS?

- Other viruses can cause FSGS: HIV-1, possibly parvovirus B19
- Polyoma viruses are renotropic
- SV40 transgenic mice develop FSGS
  MacKay, Kidney Int 1987
- FSGS incidence has risen 2-3 fold in the past 25 years, suggesting a new environmental factor
- Collapsing FSGS, a new disease appearing about 1980
Methods

- Patients: FSGS, other kidney disease, healthy volunteers
- PBMC, urinary cells
- Co-cultures with susceptible CV-1 cells:
  - T Ag staining, nested PCR of regulatory region, multiple clones sequenced

J Am Soc Nephrol, 9/02 (in press)
CV-1 Cell Infection

Volunteer d7  FSGS d7  FSGS d15
SV40-T Ag

CV-1 + Volunteer Ur

CV-1 + FSGS Ur
SV40 Regulatory Region

Early genes

T and Ag

Late genes

VP

5243 bp
SV40-776 Regulatory Region

- **Archetypal P3E1**
- **Nonarchetypal P3E2**
- **TATA**
- **Promoter**
- **Enhancer**
- **TAg Binding**
- **Sp1 binding**
- **Nested PCR primers**

RA2

RA4

RA1

RA3
Nested PCR from Healthy Volunteer Co-cultures

MW  SV 40  CV-1  N5  N6  N7  N8  N9  N9  N10  N11

315 bp
### Human SV40 Isolates: Regulatory Region Diversity

<table>
<thead>
<tr>
<th>SV40</th>
<th>GCCGAGGCGG</th>
<th>CCTCGGCCTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS20 P</td>
<td>TGCATAAATA</td>
<td>AAAAAAAATT</td>
</tr>
<tr>
<td>FSGS10 U</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>FSGS10 U</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>FSGS01 P</td>
<td>--T------</td>
<td>--------</td>
</tr>
<tr>
<td>FSGS07 P</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>FSGS08 P</td>
<td>--G-CCT-A-</td>
<td>---</td>
</tr>
<tr>
<td>CCT-A-</td>
<td>---</td>
<td></td>
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</tbody>
</table>
PCR of Positive Clones from an FSGS Patient PBMC

- SV40 1 2 3 4

316 bp

295

244

P3E2 P2E2 P2E2 P3E1
SV40 Regulatory Region Variation

- P3E2 (Non-archetypal): no mutation or 1-3 NT mutations
- P3E2: 10-12 bp mutations in T Ag binding core region
- P3E1 (Archetypal): 72 bp enhancer element deletion
- P2E2 (Novel): 21 bp promoter element deletion
## SV40 Isolation

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>PBMC</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS</td>
<td>40</td>
<td>14 (35%)</td>
<td>15/36 (42%) *</td>
</tr>
<tr>
<td>Other kidney dx</td>
<td>20</td>
<td>3 (15%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Volunteer</td>
<td>22</td>
<td>5 (23%)</td>
<td>1 (5%)</td>
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</tbody>
</table>

* * P<0.001*
Detection of other polymomaviruses by direct PCR

<table>
<thead>
<tr>
<th>Virus</th>
<th>PBMC</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK</td>
<td>3/63</td>
<td>7/61</td>
</tr>
<tr>
<td>JC</td>
<td>2/63</td>
<td>9/61</td>
</tr>
</tbody>
</table>
Lack of age effect in SV40 detection among all study subjects

<table>
<thead>
<tr>
<th>Birth date</th>
<th>PBMC</th>
<th>Urinary Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1964</td>
<td>13/50 (26%)</td>
<td>10/47 (21%)</td>
</tr>
<tr>
<td>=1964</td>
<td>9/32 (28%)</td>
<td>8/31 (26%)</td>
</tr>
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</table>

P=NS
Immunosuppression does not account for SV40 recovery in urine

<table>
<thead>
<tr>
<th>Group</th>
<th>ImmSupp</th>
<th>Not ImmSupp</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS</td>
<td>7/16</td>
<td>8/24</td>
</tr>
<tr>
<td>Other</td>
<td>2/15</td>
<td>0/5</td>
</tr>
<tr>
<td>Healthy</td>
<td>0/0</td>
<td>1/22</td>
</tr>
</tbody>
</table>

Immunosuppression: HIV-1 or immunosuppressive Rx in the preceding 3 mos
# SV40 Detection in Renal Biopsies

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N</th>
<th>SV40+</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Collapsing FSGS</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>HIV FSGS</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>All FSGS</td>
<td></td>
<td>57%</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Minimal change disease</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

\[ P < 0.02 \]
ISH: FSGS Kidney Biopsies

FSGS

HIV-FSGS
Other studies of SV40 in volunteers

- Methods: PCR, Southern blot
- Blood
  - Martini, Cancer Res 1996 16/70 (23%)
  - David, Cancer Letters 2001 18/115 (16%)
  - Butel, Lancet 2002 0/100
  - Shivapurkar, Lancet 2002 0/12
- Urine
  - Shah, J Infect Dis 1997 0/166
SV40 detection following renal transplant

- Butel, J Infect Dis 1999 4/20 pediatric tissues + SV40, including 1 Wilm’s tumor and 3 renal transplants

- NIH 5/71 renal transplant recipients developed PV nephropathy

<table>
<thead>
<tr>
<th></th>
<th>PBMC</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK</td>
<td>2/5</td>
<td>5/5</td>
</tr>
<tr>
<td>SV40</td>
<td>3/5</td>
<td>2/5</td>
</tr>
</tbody>
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Summary

- Isolation of infectious SV40 from PBMC and urine cells
- Sensitive techniques, including nested PCR and sequencing - but also risk of contamination
- Regulatory region heterogeneity argues against laboratory contamination
- SV40 recovered more frequently from urine cells of kidney patients, particularly FSGS and PV nephropathy
Conclusions

- Possible roles of SV40 in kidney
  1. Cause of glomerular injury (FSGS) and tubulitis (PV nephropathy)
  2. Bystander, activated by immunosuppression or cytokines, or more readily detected due to excretion of renal cells with injury
  3. Bystander, but the resulting tubulitis contributes to renal damage

- Frequent recovery argues for relatively common infection in the general population
## Acknowledgements

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<thead>
<tr>
<th>FSGS</th>
<th>Ronald Falk</th>
<th>Rui-Mei Li</th>
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<tr>
<td></td>
<td>Charles Jennette</td>
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<tr>
<td>Renal transplant</td>
<td>Roslyn Mannon</td>
<td>Rui-Mei Li</td>
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<td>Allen Kirk</td>
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