Malignant Transformation of Hamster Kidney Cells by BK Virus

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Primary hamster kidney cells were transformed by BK virus, a new human papovavirus. Transformed (HKBK) cells produced BK virus T antigen and induced tumors in hamsters that developed antibodies to BK virus T antigen. BK virus was rescued from HKBK cells by Sendai virus-assisted fusion with permissive cells. One out of six cell lines derived from HKBK cell-induced tumors showed the same characteristics as HKBK cells.

BK virus is a new human papovavirus isolated by Gardner et al. (6) from a patient receiving a kidney graft. Several studies have been performed recently on the biological, antigenic, and structural properties of this virus (10, 11, 18). In particular, serological investigations (6, 13, 18) have established that an antigenic relationship exists between the structural antigens as well as the T antigen of BK virus and the corresponding antigens of simian virus 40 (SV40). The identification of BK virus as a papovavirus, the wide diffusion of BK virus infection in human populations (5, 14, 16), and the presence of antibodies to BK virus T antigen in some human sera (15) raised the question of its possible role in human oncogenesis. Major and Di Mayorca (9) reported neoplastic transformation of BHK-21 cells by BK virus, but no adequate proof was given in their experiments of the specificity of transformation, since neither the virus-specific T antigen nor virus rescue was demonstrated in transformed cells.

We describe here the neoplastic transformation of hamster kidney cells by BK virus. BK virus was grown in Vero cells and titrated in human embryonic fibroblasts by the fluorescent antibody (FA) focus assay according to Aaronson and Todaro (1) and by hemagglutination of human type O erythrocytes according to Portolani et al. (14). The virus pool used in these experiments had a titer of \(3.7 \times 10^5\) FA focus-forming units per ml and \(10^8\) hemagglutinating units (HAU) per ml. Primary hamster kidney cells, obtained by trypsinization of kidneys from 72-h-old baby hamsters, were infected with 2 FA focus-forming units per cell.

After infection, cells were fed with Eagle minimal essential medium supplemented with 5% calf serum and were split twice a week. At the eighth passage control cells started to die out, whereas colonies of morphologically modified cells appeared in the infected cultures. These cells showed a fast growth rate, loss of contact inhibition, and a very high saturation density; at the 12th passage a cell line was established (HKBK) that is now in the 45th passage. HKBK cells were tested at different passage levels for virus production by assaying the hemagglutinating activity of the supernatant medium and by fluorescent staining of capsid antigens, using a specific guinea pig serum to BK virus. These tests were consistently negative.

HKBK cells \(2 \times 10^4\) at the 16th passage produced palpable tumors 20 days after inoculation in 100% of suckling and in 66% of adult hamsters (Table 1). These tumors grew fast and eventually killed the animals 2 to 3 months after the inoculation of transformed cells. Sera from all tumor-bearing hamsters were tested for the presence of antibodies to BK virus T antigen. Seventy-eight percent of sera from animals injected when suckling and 50% of sera from animals injected when adults produced a typical T antigen fluorescent staining on HKBK cells (Fig. 1). The same sera stained the T antigen in MKS-Bu100 cells transformed by SV40 (17) and in SV40-infected Vero cells. Conversely, hamster serum to the SV40 T antigen gave a typical T antigen staining on HKBK cells. Sera from tumor-bearing hamsters did not contain antibodies to the structural virus antigens, since they gave only a T antigen staining when tested on Vero cells 3 days after infection with BK virus or 2 days after infection with SV40. These sera did not contain hemagglutination-inhibiting antibodies to BK virus.

HKBK cells were fused with permissive cells,
TABLE 1. Characteristics of HKBK cells and of the six cell lines derived from HKBK cell-induced tumors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HKBK cells</th>
<th>Tumor cell lines</th>
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<tbody>
<tr>
<td>T antigen VP*</td>
<td>+98.2%</td>
<td>+6/6 (100)%</td>
</tr>
<tr>
<td>Oncogenicity</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>For suckling hamsters</td>
<td>37/37 (100)%</td>
<td>4/6(66)%</td>
</tr>
<tr>
<td>For adult hamsters</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Percentage of positive cells.
† Number of cell lines showing T antigen/number tested and percentage (in parentheses) of positive cells.
* VP, Viral coat protein antigen.
* No. of animals with tumors/no. of animals injected with HKBK cells and percentage (in parentheses) of animals with tumors.

![Image of T antigen staining of HKBK cells](image)

**Fig. 1. T antigen staining of HKBK cells.** Serum from a tumor-bearing hamster was used at the dilution of 1:2. Fluorescein-conjugated rabbit antiserum to hamster immunoglobulin G (Hyland) was diluted 1:5.

human embryonic fibroblasts, and Vero cells by means of beta-propiolactone-inactivated Sendai virus (2). In both cases, 72 h after fusion fluorescent nuclei were detected in syncyta by using a specific guinea pig serum to BK virus (Fig. 2a). The percentage of fluorescent-positive syncyta was 2.7 (Table 2). Cells from six HKBK cell-induced tumors were grown in culture and tested for T antigen and virus rescue. All were positive for T antigen when reacted with sera from tumor-bearing hamsters or with a serum to SV40 T antigen. Four of the six tumor-derived cell lines, when fused with permissive cells, exhibited fluorescent nuclei in syncyta (Fig. 2b and Table 2) after staining with a specific serum to BK virus.

Attempts to detect BK virus by hemagglutination in the supernatant medium and cell homogenate from fused cultures of HKBK and

**Fig. 2. Fluorescent nuclei in syncyta 3 days after fusion of (a) HKBK cells and (b) tumor cell line no. 2 with human embryonic fibroblasts.** Specific guinea pig serum to BK virus was diluted 1:4 and fluorescein-conjugated rabbit antiserum to guinea pig immunoglobulin G (Hyland) was diluted 1:5.

TABLE 2. Rescue of infectious BK virus from HKBK cells and from cell lines derived from HKBK cell-induced tumors

<table>
<thead>
<tr>
<th>Cells</th>
<th>Percentage of syncytia showing fluorescent nuclei*</th>
<th>Rescue of BK virus (HAU/ml)*</th>
<th>Infectivity of rescued virus for HEF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKBK Tumor cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NT†</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*72 h after fusion of transformed or tumor cells with Vero cells.
† At the 10th subculture of fused cells and upon treatment with receptor-destroying enzyme (RDE). Cells were frozen and thawed once in 4 ml of supernatant medium and subjected to sonic oscillation for 3 min. The cell homogenate was incubated overnight at 37 C with RDE at the final dilution of 1:10 and then heated at 56 C for 1 h to inactivate RDE.
* Infectivity tests were performed without heat inactivation of the RDE-treated cell homogenate. HEF, Human embryonic fibroblasts.
† NT, Not tested.
Vero cells were negative. Fused cultures were then subcultured, and the cell homogenates were tested periodically by hemagglutination directly and after treatment with receptor-destroying enzyme (Sigma Chemical Co.), which has been shown to improve the release of infectious virus from polyoma, JC, and BK virus-infected cells (3, 4, 12). Although hemagglutination was always negative when tested directly, hemagglutinating titer of 32 HAU per ml at the sixth subculture and 256 HAU per ml at the tenth subculture were detected upon treatment of the cell homogenate with receptor-destroying enzyme. The hemagglutinating activity of 8 HAU of the cell homogenate was completely inhibited by a 1:100 dilution of a specific guinea pig serum to BK virus. Eight days after infection of human embryonic fibroblasts with the hemagglutination-positive homogenate, fluorescent nuclei were detected by using the specific guinea pig serum to BK virus, and infectious virus was recovered from the supernatant medium. One out of three tumor cell lines subcultured after fusion with Vero cells gave results similar to those obtained with HKB1 cells (Table 2).

In conclusion, hamster kidney cells were transformed by BK virus; in fact, they produced the intranuclear BK virus T antigen and induced tumors in hamsters, and the tumor-bearing hamsters had antibodies to BK virus T antigen. Moreover, virus was rescued from the originally transformed cell line and from one line obtained from tumors induced in hamsters. The low frequency of rescue, in spite of the very good efficiency of fusion, was probably due to the high number of defective particles present in the virus inoculum. Indeed, even though the virus pool used for infection showed only 7.2% of coreless virions when observed by negative staining under an electron microscope, the ratio of physical particles, determined from the hemagglutinating titer (10), to FA focus-forming units was very high (10⁴ to 1). It is likely, then, that very few cells were infected and transformed by virions containing a fully infectious genome, whereas most of the cells were probably infected by defective virus particles. This may explain why essentially all BK virus-transformed cells produced T antigen, whereas only few of them were inducible when fused with permissive cells. Alternatively, a large number of transformed cells may contain a complete viral genome if the efficiency of the induction is low. This possibility is supported by the observation that the activation of the viral genome in SV40-transformed cells fused with permissive cells is limited by the inefficient excision of viral DNA from cellular DNA (7, 8).

**LITERATURE CITED**


