Stable Transformation of Mouse, Rabbit and Monkey Cells and Abortive Transformation of Human Cells by BK Virus, a Human Papovavirus

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SUMMARY

Semi-permissive mouse, rabbit and monkey cells were stably transformed by BK virus (BKV). The specificity of transformation was demonstrated by the presence of BKV tumour (T) antigen in nuclei of transformed cells and by virus rescue with Sendai virus-mediated fusion or transfection. Two out of seven BKV-transformed cell lines were oncogenic. Permissive human cells were only abortively transformed by BKV, since morphologically modified cells persisted in culture for a few passages and eventually died.

BK virus (BKV) is a human papovavirus isolated from urine of a renal transplant recipient (Gardner et al. 1971). Several lines of evidence suggest that human papovaviruses may be related to human tumours (Takemoto et al. 1974; Corallini et al. 1976; Fiori & Di Mayorca, 1976). However, the relevance of BKV to human oncogenesis rests on preliminary demonstration of its oncogenic potential for experimental animals and transforming ability for cells in culture. A number of reports established the oncogenicity of BKV for rodents, with different incidence of tumours according to route of inoculation and amount of virus administered (Shah, Daniel & Strandberg, 1975; Van der Noordaa, 1976; Corallini et al. 1977). Transformation of non-permissive hamster and rat cells was obtained after infection with BKV or BKV-DNA (Major & Di Mayorca, 1973; Portolani, Barbanti-Brodano & La Placa, 1975; Takemoto & Martin, 1976; Tanaka, Koprowski & Iwaski, 1976; Van der Noordaa, 1976), but no transformation of cells from other species has been described. We report here stable transformation of mouse, rabbit and monkey cells and abortive transformation of human cells by BKV.

Prototype BKV, kindly supplied by Dr Sylvia D. Gardner, was grown in VERO cells as described previously (Maraldi et al. 1975). Virus was titrated by haemagglutination of type O human erythrocytes (Portolani et al. 1974) and by the fluorescent antibody (FA) focus assay in human embryonic fibroblasts (HEF) according to Aaronson & Todaro (1970). Mouse cells were obtained by trypsinization of kidneys of 8-day-old mice and from whole embryos either of Swiss or BALB/c strains. Rabbit cells were obtained from kidneys, brain and liver of New Zealand white rabbits and monkey cells by trypsinization of rhesus monkey kidneys. HEF were derived from skin and muscles of a 4-month-old embryo.

For transformation assays, monolayers of semi-confluent cells were infected with BKV at the second passage in culture, except HEF which were employed between the 8th and 14th passages. An input multiplicity of 10 FA focus forming units (FA f.f.u.) per cell was used in all experiments. When cells reached confluence the cultures were divided in the ratio of 1:3. BKV tumour (T) antigen was detected by immunofluorescence (IF) with a specific serum obtained from hamsters bearing tumours induced by BKV-transformed hamster cells (Portolani et al. 1975). Virus coat protein (VP) antigens were detected by IF with a specific hyperimmune guinea-pig serum (Portolani et al. 1975). To rescue BKV from
BKV-transformed cells, an equal amount of transformed and permissive cells (VERO or HEF) were fused with beta-propiolactone-inactivated Sendai virus (Barbanti-Brodano, Possati & La Placa, 1971). Activation of BKV was monitored by IF staining of heterokaryons for BKV VP antigens 3 days after fusion and by testing the haemagglutinating activity of cell homogenates at different passages of fused cultures. Cell homogenates were sometimes treated with receptor destroying enzyme (RDE) in order to improve release of virus from cell debris. DNA was extracted from transformed cells with phenol and sodium dodecyl sulphate as described previously (Swetley et al. 1969), treated twice for 30 min at 37 °C with 50 μg/ml pancreatic ribonuclease (boiled for 10 min) and precipitated with ethanol. HEF and VERO cells were transfected with DNA from BKV-transformed cells at a rate of 50 μg DNA and 100 μg DEAE-dextran per 10^6 cells (Boyd & Butel, 1972). As a positive control, HEF and VERO cells were also infected with BKV-DNA (2.5 μg/10^6 cells, infectious titre 10^4 FA f.f.u./μg) extracted from purified virions (Swetley et al. 1969).

A few days after infection moderate cytopathology appeared in mouse, rabbit and monkey infected cells. A low level of haemagglutinating activity (8 to 32 HAU/ml) was found in the culture medium and BKV VP antigens were detected in the nuclei of 12 to 28 % infected cells. Between the 3rd and 8th passages post infection (p.i.), while several cells were dying due to virus induced lysis, other cells were actively dividing and colonies of morphologically modified cells started to appear in the infected cultures. The morphologically transformed mouse and rabbit cells showed an epithelial- or spindle-like shape, complete loss of contact inhibition and a fast growth rate in 10% serum (doubling time 18 h for mouse cells and 22 h for rabbit cells). In addition they were able to grow in 1% serum (doubling time 34 h for mouse cells and 41 h for rabbit cells). They reached a high saturation density both in 10% and in 1% serum (4.8 x 10^4 cells/cm^2 and 3.4 x 10^4 cells/cm^2 for mouse cells, 4.4 x 10^4 cells/cm^2 and 3.1 x 10^4 cells/cm^2 for rabbit cells). Transformed monkey cells grew more slowly in 10% serum (doubling time 34 h) and in 1% serum (doubling time 56 h), reached a lower saturation density (3.0 x 10^4 cells/cm^2 in 10% serum) and showed only a partial loss of contact inhibition. At the 10th passage, p.i. cell lines were established which are now over the 100th passage. Mouse, rabbit and monkey cell lines were repeatedly tested between the 20th and 30th passages p.i. for the presence of haemagglutinating activity in the culture medium and VP antigens in cell nuclei. These tests were consistently negative.

Human cells behaved in a different way in that cell lysis was more extensive and a small number of cells had remained in the infected cultures at the 6th to 8th passage p.i. After a period of crisis (20 to 30 days) when cells did not divide, colonies of epithelial-like cells, morphologically different from uninfected control cells, started to appear in the infected cultures (Fig. 1a and b). When cells in colonies were dispersed by trypsin they lost the polygonal shape of transformed cells and reverted to their original fibroblastic appearance. Epithelial-like cells reappeared after some replication cycles, again producing colonies in the infected cultures. Reversion from epithelial to fibroblastic morphology and vice versa was observed in cultures of BKV-infected HEF every time cells in colonies were dispersed. However, after a few passages in culture, morphologically transformed cells invariably died out. Haemagglutinating activity and VP antigens (in nuclei of about 10% of cells) were constantly detected in BKV-infected HEF until death of the cells. Fourteen experiments were performed to obtain a stable cell line from BKV-infected HEF, including use of u.v.-irradiated BKV (surface dose 720 erg/cm^2/s, 4 to 16 min of irradiation) and anti-BKV specific serum (1 % in the culture medium, neutralizing titre 1:2560); all the experiments ended in lysis of the infected cultures, virus irradiation and BKV specific serum only.
Fig. 1. (a) A colony of morphologically transformed HEF at the 13th passage after infection with BKV. (b) A control culture of HEF at the same passage level. (c) Nuclei containing T antigen in BKV-transformed monkey cells and (d) HEF growing in colonies.
Table 1  *Characteristics of mouse, rabbit, monkey and human cells transformed by BKV*

<table>
<thead>
<tr>
<th>Cells</th>
<th>Production of VP antigen and virus*</th>
<th>Percentage of T antigen-positive cells</th>
<th>Percentage of VP-positive heterokaryons†</th>
<th>HAU/ml in cell homogenates</th>
<th>Transfection HAU/ml in cell homogenates</th>
<th>Oncogenicity</th>
</tr>
</thead>
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<tr>
<td>Mouse kidney Swiss</td>
<td>-</td>
<td>96</td>
<td>0</td>
<td>0†</td>
<td>64 (8)$</td>
<td>21/2311</td>
</tr>
<tr>
<td>Mouse embryo Swiss</td>
<td>-</td>
<td>98</td>
<td>2.3</td>
<td>128‡ (10)$§</td>
<td>ND†</td>
<td>8/10</td>
</tr>
<tr>
<td>Mouse kidney BALB/c</td>
<td>-</td>
<td>98</td>
<td>0.9</td>
<td>64‡ (13)</td>
<td>ND</td>
<td>0/15**†† 0/17***††</td>
</tr>
<tr>
<td>Mouse embryo BALB/c</td>
<td>-</td>
<td>97</td>
<td>3.1</td>
<td>256 (13)</td>
<td>16 (6)</td>
<td>0/18**†† 0/17***††</td>
</tr>
<tr>
<td>Rabbit kidney</td>
<td>-</td>
<td>100</td>
<td>0.5</td>
<td>32 (5)</td>
<td>ND</td>
<td>0/13**</td>
</tr>
<tr>
<td>Rabbit brain</td>
<td>-</td>
<td>95</td>
<td>0</td>
<td>0‡</td>
<td>ND</td>
<td>0/12**</td>
</tr>
<tr>
<td>Rabbit liver</td>
<td>-</td>
<td>75</td>
<td>1.0</td>
<td>32 (5)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Monkey kidney</td>
<td>-</td>
<td>100</td>
<td>0</td>
<td>0‡</td>
<td>16 (6)</td>
<td>0/7**</td>
</tr>
<tr>
<td>Human embryo fibroblasts</td>
<td>+</td>
<td>5 to 40</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Tested between the 20th and 30th passages in the established cell lines.
† Detected 3 to 5 days after fusion.
‡ Cell homogenates were treated with RDE.
§ In parentheses, the number of culture passages after fusion or transfection when haemagglutinating activity was detected.
¶ ND = not done.
** 1 to 1.5 x 10⁷ cells per animal were inoculated.
†† Cells were inoculated in newborn BALB/c mice.
††† Cells were inoculated in the cheek pouch of adult hamsters immunosuppressed with anti-lymphocyte serum.
delaying the time of cell death. Attempts to obtain stable transformation of BKV-infected human embryo kidney cells and cells from normal human uterine cervix also failed.

To demonstrate the specificity of transformation, transformed cells were tested for the presence of BKV T antigen and virus rescue. Approximately 100% of transformed cells from established cell lines of mouse, rabbit and monkey origin tested by IF between the 20th and 30th passages p.i. showed a granular or reticular nuclear fluorescence typical of papovavirus T antigen (Table I and Fig. 1 c). BKV T antigen was detected in nuclei of 5 to 40% infected HEF depending on different passages (Table I and Fig. 1 d). BKV was rescued from three of the mouse cell lines and from rabbit kidney and liver cells by fusion with HEF or VERO cells (Table I). BKV rescue was obtained by transfection of HEF and VERO cells with DNA from monkey and Swiss mouse kidney transformed cells, which gave no rescue after fusion (Table I). Virus rescued either by fusion or by transfection agglutinated type O human erythrocytes and haemagglutinating activity was inhibited by a specific serum to BKV VP antigens. Virus DNA was infectious as haemagglutinating activity (128 HAU/ml) was found in cell homogenates of HEF two passages after infection with BKV-DNA.

Mouse cells of Swiss strain produced tumours when injected subcutaneously in newborn Swiss mice. Tumours reached a size of about 5 mm 20 days after inoculation of BKV-transformed cells and then slowly regressed. Mouse cells of BALB/c strain did not induce tumours either when inoculated subcutaneously in newborn syngeneic mice or when injected into the cheek pouch of hamsters immunosuppressed with anti-lymphocyte serum. Rabbit cells inoculated subcutaneously in newborn New Zealand rabbits and monkey cells inoculated in newborn hamsters (Table 1) were also non-oncogenic.

These results indicate that it is possible to transform semi-permissive cells with BKV and extend previous demonstrations of the transforming ability of BKV for non-permissive cells. The specificity of transformation was proved by the presence of BKV T antigen in the nuclei of transformed cells and by virus rescue obtained by Sendai virus-mediated fusion or transfection. Failure to induce progressively growing tumours with Swiss mouse, rabbit and monkey transformed cells may depend on immunological rejection by heterologous hosts, whereas lack of oncogenicity of BALB/c mouse transformed cells in syngeneic animals has no satisfactory explanation. The transient appearance of cells with morphologically transformed phenotype and synthesizing T antigen in cultures of BKV-infected HEF is reminiscent of the abortive transformation described with other papovaviruses (Stoker, 1968). At present however it is not clear whether failure to obtain stable transformation of HEF is due to lack of integration of the virus genome into the cell genome, as probably happens in abortive transformation, or to the peculiar permissiveness of HEF to lytic infection by BKV. Similar results with human fibroblasts infected by BKV were recently reported by Shah et al. (1976). Since BKV has more difficulty than the other papovaviruses, polyoma and simian virus 40, in transforming permissive cells, more efforts and the use of special artifacts will be needed to induce stable transformation of permissive cells with BKV.

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Note added in proof. While this article was in the press, a paper was published [Mason, D. H. & Takemoto, K. K. (1977). International Journal of Cancer 19, 391–395] which reported results similar to ours with MM virus, a human papovavirus closely related to BKV.

REFERENCES


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