J. gen. Virol. (1988), **69**, 2671–2679. *Printed in Great Britain*

Key words: *BK virus/oncogene, human c-Ha-*ras*/intracerebral tumours*

Cooperation in Oncogenesis between BK Virus Early Region Gene and the Activated Human c-Harvey *ras* Oncogene

By A. CORALLINI,[1] M. PAGNANI,[1] A. CAPUTO,[1] M. NEGRINI,[1] G. ALTAVILLA,[2] L. CATOZZI[3] AND G. BARBANTI-BRODANO[1]*

Institutes of [1]*Microbiology,* [2]*Pathologic Anatomy and* [3]*Radiology, School of Medicine, University of Ferrara, Via Luigi Borsari 46, I-44100 Ferrara, Italy*

(*Accepted 7 May 1988*)

SUMMARY

Rapidly growing, undifferentiated brain tumours were induced in newborn Syrian hamsters by intracerebral inoculation of a recombinant DNA (pBK/c-*ras*A) carrying the BK virus (BKV) early region gene and the activated human c-Harvey-*ras* (c-Ha-*ras*) oncogene. Neither of the two genes inoculated alone nor recombinant DNA of the BKV early region gene and the normal human c-Ha-*ras* proto-oncogene were tumourigenic. Tumour-derived cell lines propagated in culture were immortalized and had growth characteristics consistent with a fully transformed phenotype. Tumours and tumour cell lines contained pBK/c-*ras*A sequences integrated into cellular DNA and expressed BKV- and c-Ha-*ras*-specific transcripts as well as BKV T antigen and c-Ha-*ras* p21. These findings are discussed in relation to a possible cooperation or synergism between BKV and cellular oncogenes in human neoplasia.

Human viruses displaying transforming activity and experimental oncogenicity are widely investigated for their potential association with human tumours. BK virus (BKV) is a human papovavirus ubiquitous in human populations. Primary infection generally occurs during childhood and is followed by a persistent, latent infection which is reactivated under conditions of impaired immunological response. BKV and BKV DNA transform hamster, rat, mouse, rabbit, monkey and human cells *in vitro*. BKV is highly oncogenic when inoculated intracerebrally (i.c.) or intravenously (i.v.) into immunosuppressed or immunocompetent hamsters, causing tumours in 73 to 88% of animals. The virus has a marked tropism for specific organs, since tumours observed in infected animals belong essentially to only three histotypes: ependymomas and choroid plexus papillomas, tumours of pancreatic islets and osteosarcomas (for review, see Yoshiike & Takemoto, 1986). BKV DNA is not oncogenic when inoculated subcutaneously (s.c.) or i.v., but induces ependymomas at low frequency (5·1%) when injected i.c. in hamsters (Corallini *et al.*, 1982).

BKV DNA, RNA and T antigen have been detected in human brain tumours and tumours of pancreatic islets (Caputo *et al.*, 1983; Pagnani *et al.*, 1986; Corallini *et al.*, 1987). Since BKV DNA in the neoplastic tissue is free, BKV was recovered from tumours by transfection of total tumour DNA into human embryonic fibroblasts. Restriction endonuclease mapping of isolated viruses showed that they are all similar to each other and different from the wild-type in that they bear an insertion and a deletion in the early region of the genome. Nucleotide sequence analysis of one of these variants (Pagnani *et al.*, 1986) showed that the inserted nucleotides can form an insertion sequence (IS)-like structure (Finnegan, 1985). This transposable element might be involved in oncogenesis by excision from viral DNA and insertion into the cell genome, thereby inducing a mutagenic effect or activating the expression of cellular oncogenes. The latter hypothesis is supported by the observation that the IS contains in its loop two of the three viral transcriptional enhancers.

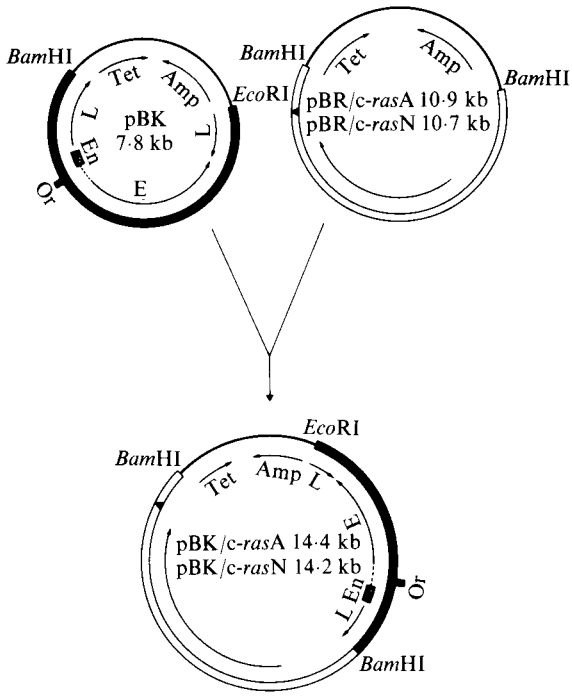

Fig. 1. Recombinant DNA vectors pBK, pBR/c-*ras*A, pBR/c-*ras*N, pBK/c-*ras*A and pBK/c-*ras*N. Black and white boxes and thick lines represent sequences of BKV early region, c-Ha-*ras*A or c-Ha-*ras*N and pML or pBR322 respectively. Compared to c-Ha-*ras*A, the c-Ha-*ras*N fragment has a 200 nucleotide deletion (▲) comprising cellular sequences 3′ of c-Ha-*ras* sequences. Internal lines indicate length of transcripts and arrows indicate direction of transcription. Amp and Tet mark the genes for ampicillin and tetracycline resistance in pML and pBR322. En, BKV transcriptional enhancer elements; Or, BKV origin of DNA replication; *Bam*HI and *Eco*RI indicate enzyme cleavage sites. E and L mark the early and late regions, respectively.

Transformation by papovaviruses is induced and maintained by T antigen (Tegtmeyer, 1980). Although human cells transformed by BKV or BKV early region DNA and expressing BKV T antigen grow as immortalized cell lines, they never show a completely transformed phenotype (Purchio & Fareed, 1979; Takemoto *et al.*, 1979; Grossi *et al.*, 1982; A. Corallini, unpublished observations). Human embryo kidney cells transfected with a recombinant plasmid containing the BKV wild-type early region and the adenovirus 12 early region 1A gene are fully transformed and grow as a continuous cell line (Vasavada *et al.*, 1986), which suggests that, at least in human cells, BKV T antigen can cause only a partially transformed phenotype and must interact with other oncogenic functions to induce complete transformation. Therefore we established an experimental model to evaluate the possible cooperation in tumourigenicity between BKV and cellular oncogenes. For this purpose we constructed recombinant DNA molecules containing the BKV early region and either the human activated c-Ha-*ras* oncogene (c-*ras*A) or the normal c-Ha-*ras* proto-oncogene (c-*ras*N) and assayed their oncogenic potential in hamsters. c-*ras*A and c-*ras*N are genomic clones derived from T24 human bladder carcinoma cells or normal human foetal liver respectively. They are essentially identical in their restriction maps and nucleotide sequences, except that c-*ras*A has a substitution, responsible for its activation, in the 12th codon of the first exon and c-*ras*N has a deletion of 200 nucleotides in the 3′ portion of the DNA segment, outside the coding sequences of c-Ha-*ras*.

Techniques for DNA and RNA hybridization as well as immunoblotting, immunoperoxidase staining and indirect immunofluorescence have been described (Pagnani *et al.*, 1986; Corallini *et al.*, 1987). Recombinant DNAs (Fig. 1) were prepared according to standard cloning methods

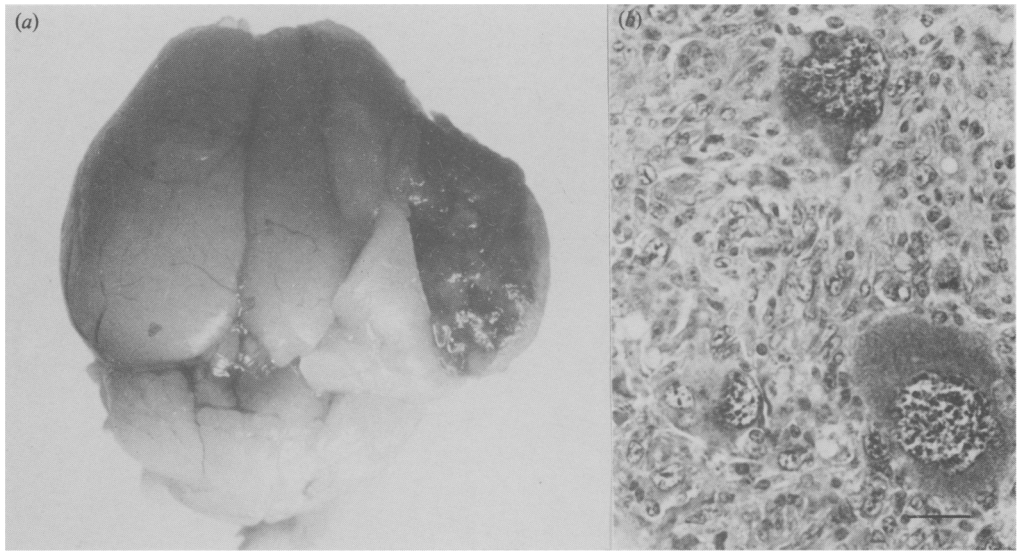

Fig. 2. Hamster brain tumour induced by pBK/c-*ras*A. (*a*) Macroscopic appearance showing a large neoplastic mass projecting from the brain. (*b*) Histology (Mayer's haematoxylin) and immunoperoxidase staining of c-*ras* p21 with RAP-5 monoclonal antibody. The tumour consists of highly atypical, polymorphic cells with frequent giant cells. The p21-reacting product appears as a dark substance concentrated in the cytoplasm and plasma membrane of tumour cells. Bar marker represents 50 μm.

(Maniatis *et al.*, 1982). The vector pBK (Milanesi *et al.*, 1984), containing the BKV wild-type (Gardner strain) early region, was constructed by ligating the large *Bam*HI/*Eco*RI fragments of the BKV genome and pML, a deletion derivative of pBR322 (Lusky & Botchan, 1981). c-*ras*A and c-*ras*N were first cloned in lambda Charon 4A phage (Santos *et al.*, 1982), then subcloned into the unique *Bam*HI site of the plasmid pBR322 (pBR/c-*ras*A and pBR/c-*ras*N). pBK/c-*ras*A and pBK/c-*ras*N were constructed by inserting the 6·6 kb *Bam*HI fragment, containing c-*ras*A, or the 6·4 kb *Bam*HI fragment, containing c-*ras*N, into the unique *Bam*HI site of pBK. In these experiments we used recombinants of c-*ras*A and c-*ras*N with the same orientation of transcription and promoter position relative to BKV transcriptional enhancers.

In the first experiment 12 newborn hamsters (less than 24 h old) were inoculated i.c. with 2 μg of pBK/c-*ras*A DNA dissolved in phosphate-buffered saline (20 μl per animal). Eight of these hamsters (66·6%) developed brain tumours with a latency period of 2 to 8 weeks. In a second confirmatory experiment groups of 21 newborn hamsters were inoculated i.c. with BKV DNA, pBK, pBK/c-*ras*A, pBK/c-*ras*N, pBR/c-*ras*A or pBR/c-*ras*N. Each hamster in the first group received 1 μg of BKV DNA. Animals in the other groups were injected with equimolar proportions of the recombinants with respect to this amount of BKV DNA. Between 2 and 6 weeks after inoculation 16 (76·2%) of the animals inoculated with pBK/c-*ras*A developed brain tumours. None of the animals in the other groups had tumours after an observation period of 1 year. Tumours had a rapid exophytic growth, protruding from the brain surface (Fig. 2*a*). Histologically they were all similar, consisting of highly atypical, polymorphic cells (Fig. 2*b*). By immunohistochemical analysis they were negative for desmin, glial acidic fibrillary protein, S-100 protein and cytokeratin, whereas they reacted with a monoclonal antibody to vimentin, a typical marker of mesenchymal cells (Osborn & Weber, 1983). They were therefore diagnosed as undifferentiated pleomorphic sarcomas. Cell lines were derived from tumours explanted *in vitro* and cultured in Dulbecco's minimum essential medium supplemented with 10% foetal bovine serum. They were propagated beyond 200 population doublings and showed high saturation density, short doubling time, low serum dependence, high cloning efficiency on plastic and in agar and a disorganized and fragmented pattern of actin cables when tested with rhodamine-conjugated phalloidin (Wulf *et al.*, 1979) (data not shown).

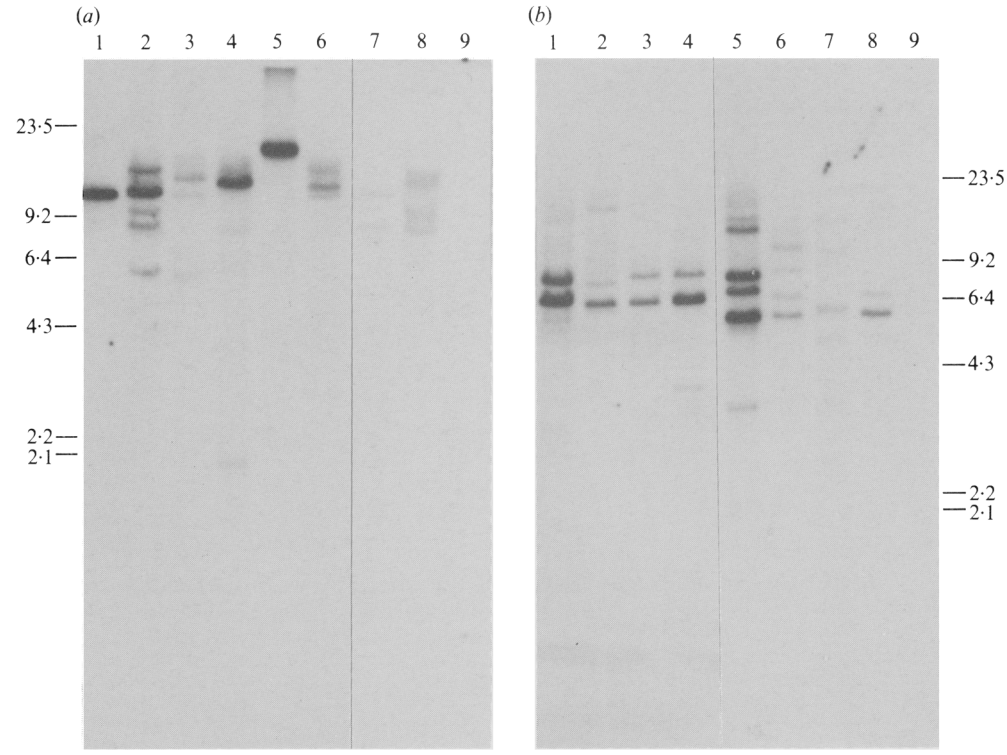

Fig. 3. Southern blot hybridization of *Eco*RI-digested (*a*) and *Bam*HI-digested (*b*) total cellular DNA (20 μg per lane) from hamster brain tumours to a ^{32}P-labelled pBK/c-*ras*A probe (sp. act. 4·5 × 10^8 c.p.m./μg). Lanes 1 contain pBK/c-*ras*A control DNA (five genome equivalents per diploid cell genome). *Eco*RI digestion (*a*) generated a full-length linear molecule (14·4 kb); *Bam*HI digestion (*b*) produced two bands corresponding to pBK (7·8 kb) and to c-Ha-*ras*A (6·6 kb). Lanes 2 to 8 contain DNA from hamster brain tumours 518, 524, 538, 539, 548, 553 and 556; lanes 9 contain DNA from early passage normal hamster embryo cells. Autoradiograms were exposed for 24 h at −70 °C. Sizes (kb) are indicated alongside.

Total cellular DNA from brain tumours and tumour-derived cell lines was hybridized to a ^{32}P-labelled pBK/c-*ras*A probe. Cellular DNA had been previously digested with *Eco*RI and *Bam*HI which have one and two cleavage sites respectively in pBK/c-*ras*A (Fig. 1). Both *Eco*RI and *Bam*HI hybridization patterns (Fig. 3*a* and *b*) showed that all tumours contained pBK/c-*ras*A integrated sequences, as either single or tandem insertions. The latter are indicated by bands comigrating with full-length linear pBK/c-*ras*A (14·4 kb) in *Eco*RI-digested cellular DNAs or with pBK (7·8 kb) and c-Ha-*ras*A (6·6 kb) in *Bam*HI-cleaved DNAs. Tumours contained one to six genome equivalents of exogenous DNA per diploid cell genome, as determined by densitometric scanning of hybridization bands. The integration pattern was different in each tumour. Moreover, after a short time of autoradiographic exposure no hybridization bands appeared in DNA from early passage normal hamster embryo cells (Fig. 3*a* and *b*, lanes 9), indicating that in tumours hybridization occurs primarily with the transfected DNA and not with the endogenous c-*ras* sequences of the hamster host cells. *Eco*RI and *Bam*HI hybridization profiles of tumour cell lines were very similar to those of the primary tumours from which each cell line originated (data not shown).

The 1·2 kb c-Ha-*ras*-specific transcript (Yaswen *et al.*, 1985) was detected in tumour cell lines (Fig. 4*a*, lanes 2 to 6) by hybridization of total cytoplasmic RNA to the c-Ha-*ras* cDNA probe (Fasano *et al.*, 1983), whereas BHK cells, a spontaneously transformed hamster cell line, were negative (Fig. 4*a*, lane 1). Two major bands of 2·3 and 2·6 kb, corresponding in size to large T

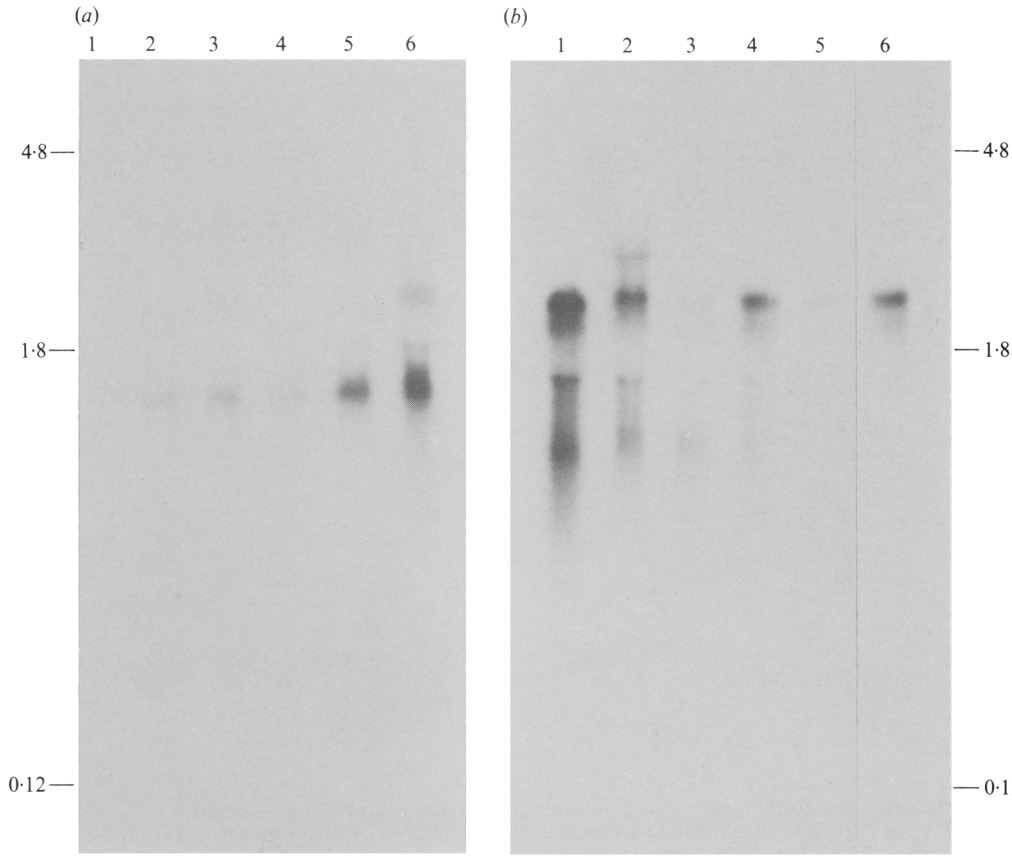

Fig. 4. Northern blot analysis to detect c-Ha-*ras* and BKV early region transcripts. Samples of total cytoplasmic RNA (20 μg per lane) were analysed by agarose gel electrophoresis and hybridization to the ^{32}P-labelled 0·8 kb c-Ha-*ras* cDNA (sp. act. 3·2 × 10^8 c.p.m./μg) (*a*) or BKV DNA 3·2 kb *Pvu*II fragment (sp. act. 5·5 × 10^8 c.p.m./μg) containing the entire early region (*b*). In (*a*) lane 1 contains RNA from BHK cells, a spontaneously transformed hamster cell line; in (*b*) lane 1 contains RNA from L578 cells transformed by BKV DNA. In (*a*) and (*b*) lanes 2 to 6 contain RNA from tumour cell lines 518, 524, 538, 539 and 553. M_r markers (28S, 18S and 5S rRNAs) are indicated with their sizes in kb.

antigen 19S and small t antigen 20S mRNAs (Seif *et al.*, 1979), were detected by hybridization of total cytoplasmic RNA from tumour cell lines to the ^{32}P-labelled BKV DNA 3·2 kb *Pvu*II fragment, containing the entire early region (Fig. 4*b*, lanes 2 to 6). These bands comigrated with the hybridization bands observed in L578 cells transformed by BKV DNA (Fig. 4*b*, lane 1). Bands that migrated faster may represent transcripts that were initiated at the BKV early promoter and terminated at a cellular stop signal.

c-Ha-*ras* p21 was detected by immunoperoxidase staining with RAP-5 monoclonal antibody (Thor *et al.*, 1984) in all pBK/c-*ras*A-induced brain tumours. Normal hamster brain and 43 out of 45 BKV-induced tumours (22 ependymomas, 13 tumours of pancreatic islets, five osteosarcomas and five subendocardial sarcomas from previous experiments) which were used as controls were negative, whereas two control tumours showed a low expression of p21, probably derived from endogenous c-*ras* genes. The immunoperoxidase reaction was localized in the cytoplasm and cytoplasmic membrane of virtually all tumour cells (Fig. 2*b*). A prominent band of M_r 21 000 was detected by immunoblotting when anti-Ha-*ras* p21 Y13-259 monoclonal antibody (Furth *et al.*, 1982) was reacted with lysates of tumours and tumour cell lines (Fig. 5, lanes 2 to 6 and 8 to 13). Normal hamster skin, chosen as a control owing to the mesenchymal origin of tumours, was negative (Fig. 5, lane 1) and spontaneously or BKV-transformed hamster

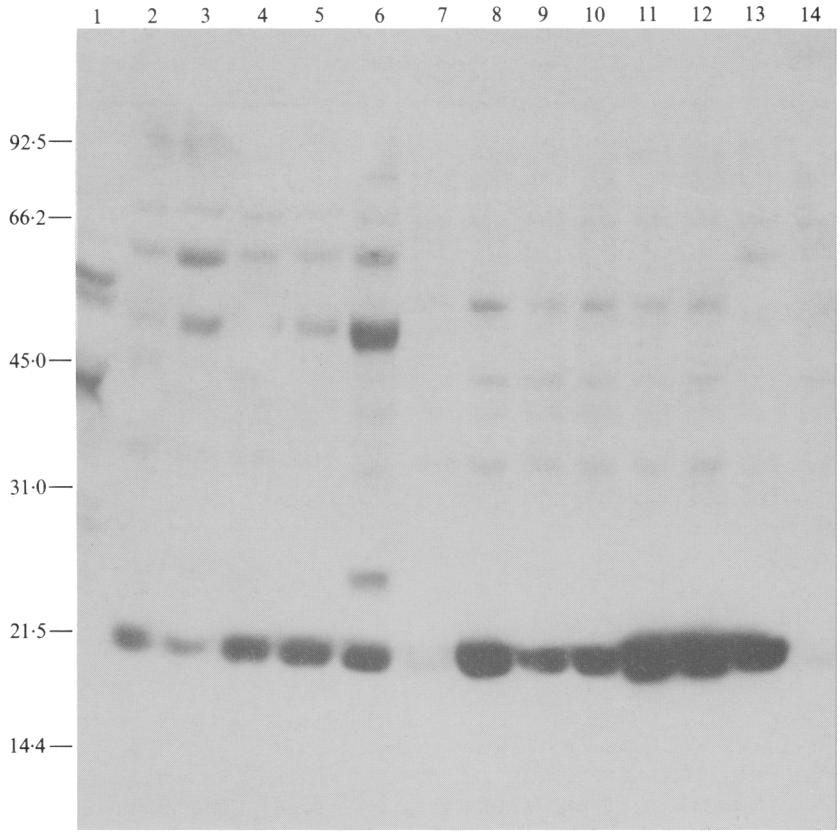

Fig. 5. Detection of c-*ras* p21 by immunoblotting with Y13-259 monoclonal antibody. Lane 1, normal hamster skin; lanes 2 to 6, hamster brain sarcomas 518, 524, 538, 539 and 553; lane 7, BHK cells (see legend to Fig. 4); lanes 8 to 13, cell lines derived from brain tumours 518, 524, 538, 539, 548 and 553; lane 14, L578 cells (see legend to Fig. 4). M_r values ($\times 10^{-3}$) of marker proteins (phosphorylase b, 92·5; bovine serum albumin, 66·2; ovalbumin, 45·0; carbonic anhydrase, 31·0; soybean trypsin inhibitor, 21·5; lysozyme, 14·4) are indicated on the left.

cells showed only a weak p21 expression (Fig. 5, lanes 7 and 14), suggesting that in tumour cells p21 is expressed mostly by the acquired c-Ha-*ras* oncogene. BKV T antigen was detected by indirect immunofluorescence in cell nuclei of all primary tumours (data not shown) and tumour cell lines (Fig. 6). Eight out of 10 sera from tumour-bearing hamsters, tested by immunofluorescence, were found to contain antibodies to BKV T antigen.

All brain tumours induced by pBK/c-*ras*A were similar in histotype and showed the histochemical markers of undifferentiated sarcomas, suggesting that pBK/c-*ras*A displays tissue-specific expression and mesenchymal cells are the primary target for its tumourigenicity. According to the multifactorial concept of carcinogenesis, these findings indicate that in hamsters cooperation of the BKV early region gene with another oncogenic function is required for neoplastic transformation of mesenchymal cells. This conclusion is in agreement with the lack or low degree of BKV DNA oncogenicity after s.c. or i.c. inoculation in hamsters (Corallini *et al.*, 1982) and the inability of activated c-Has-*ras* to induce complete transformation of primary rodent cells (Land *et al.*, 1983; Ruley, 1983; Newbold & Overell, 1983). Cell lines derived from tumours and propagated in culture were immortalized and showed a fully transformed phenotype, indicating that the combination of BKV and c-Ha-*ras*A oncogenic functions is able to maintain stable transformation.

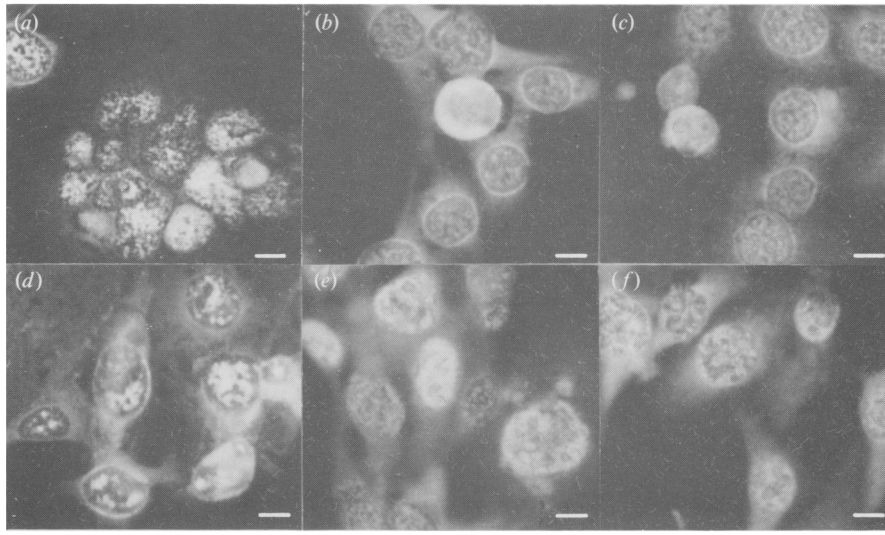

Fig. 6. Detection of BKV T antigen by indirect immunofluorescence in cell lines derived from hamster brain tumours 518 (*a*), 524 (*b*), 538 (*c*) 539 (*d*), 548 (*e*) and 553 (*f*). Bar markers represent 5 μm.

As over-expression of the activated c-Ha-*ras* oncogene induces transformation of early passage rodent cells *in vitro* (Spandidos & Wilkie, 1984), we cannot exclude the possibility that stimulation of c-Ha-*ras* expression by *cis*-acting BKV transcriptional enhancers contributes to the oncogenic effect, although it seems unlikely for the following reasons. Over-expression of c-Ha-*ras* was obtained by closely linking the very strong promoter–enhancer element of Moloney murine sarcoma virus (Laimins *et al.*, 1982) to the oncogene in sense orientation, whereas BKV enhancer repeats are weak in rodent cells (Rosenthal *et al.*, 1983). In addition, in our recombinants BKV enhancers are positioned about 4·5 kb upstream of the c-Ha-*ras* promoter and in antisense orientation relative to the c-Ha-*ras* direction of transcription (Fig. 1), a situation producing a low enhancing effect (Rosenthal *et al.*, 1983).

The mechanism of the interaction between the two transforming genes was clarified by cotransfection of primary hamster embryo cells with BKV DNA and either pBR/c-*ras*A or pBR/c-*ras*N (Pagnani *et al.*, 1988). Cotransfected molecules produced a number of foci comparable to that produced by pBK/c-*ras*A and pBK/c-*ras*N respectively, demonstrating that the BKV early region gene and c-Ha-*ras* independently express functions that cooperate in transformation. Moreover, transfection of BKV DNA-transformed hamster embryo cells with pBR/c-*ras*A and of pBR/c-*ras*A-transformed cells with BKV DNA induced foci of rapidly growing, morphologically altered cells. These results indicate that the second transfected molecule is able to confer additional phenotypic properties to transformed cells and that a fully transformed phenotype can be caused by the BKV early region gene and c-Ha-*ras* acting separately in two steps. Cell lines obtained from cotransfected and supertransfected foci expressed high levels of c-*ras* p21 and produced BKV T antigen. As to the nature of cooperation between the two transforming genes, since c-Ha-*ras*A confers morphological alteration and anchorage independence to early passage rodent cells and can be complemented by the immortalizing functions of polyoma virus large T antigen (Land *et al.*, 1983), in our experimental model immortalization may involve BKV large T antigen.

Cooperation of wild-type BKV and oncogenes in induction of the neoplastic phenotype may be relevant to the possible role of BKV in natural human oncogenesis. Moreover, BKV variants associated with human tumours contain in the early region a putative transposable element including in its sequence two viral enhancer repeats (Pagnani *et al.*, 1986). Several cellular oncogenes are involved in different steps of human carcinogenesis (Slamon *et al.*, 1984; Bishop,

1987) and could be deregulated or activated through a mutagenic effect or enhancement of transcription (Varmus, 1984) by insertion of this specific transposable sequence. Therefore it would seem to be worthwhile to explore further the interaction of wild-type BKV and its variants with other cellular oncogenes, in order to gain more information on the possible cooperation or synergism between viral and oncogene functions in human neoplasia.

We thank A. Bevilacqua and M. Bonazzi for animal care, technical assistance and typing the manuscript. We are grateful to Professor T. Wieland for the gift of rhodamine-conjugated phalloidin and to Dr J. Schlom for the gift of RAP-5 monoclonal antibody. This study was supported by the Italian National Research Council (Special Projects 'Oncology' and 'Genetic Engineering and Molecular Basis of Hereditary Diseases') and by the Associazione Italiana per la Ricerca sul Cancro.

REFERENCES

BISHOP, J. M. (1987). The molecular genetics of cancer. *Science* **235**, 305–311.

CAPUTO, A., CORALLINI, A., GROSSI, M. P., CARRA', L., BALBONI, P. G., NEGRINI, M., MILANESI, G., FEDERSPIL, G. & BARBANTI-BRODANO, G. (1983). Episomal DNA of a BK virus variant in a human insulinoma. *Journal of Medical Virology* **12**, 37–49.

CORALLINI, A., ALTAVILLA, G., CARRA', L., GROSSI, M. P., FEDERSPIL, G., CAPUTO, A., NEGRINI, M. & BARBANTI-BRODANO, G. (1982). Oncogenicity of BK virus for immunosuppressed hamsters. *Archives of Virology* **73**, 243–253.

CORALLINI, A., PAGNANI, M., VIADANA, P., SILINI, E., MOTTES, M., MILANESI, G., GERNA, G., VETTOR, R., TRAPELLA, G., SILVANI, V., GAIST, G. & BARBANTI-BRODANO, G. (1987). Association of BK virus with human brain tumors and tumors of pancreatic islets. *International Journal of Cancer* **39**, 60–67.

FASANO, O., TAPAROWSKY, E., FIDDES, J., WIGLER, M. & GOLDFARB, M. (1983). Sequence and structure of the coding region of the human H-*ras*-1 gene from T24 bladder carcinoma cells. *Journal of Molecular and Applied Genetics* **2**, 173–180.

FINNEGAN, D. J. (1985). Transposable elements in eukaryotes. *International Review of Cytology* **93**, 281–326.

FURTH, M. E., DAVIS, L. J., FLEURDELYS, B. & SCOLNICK, E. M. (1982). Monoclonal antibodies to the p21 products of the transforming gene of Harvey murine sarcoma virus and of the cellular *ras* gene family. *Journal of Virology* **43**, 294–304.

GROSSI, M. P., CAPUTO, A., MENEGUZZI, G., CORALLINI, A., CARRA', L., PORTOLANI, M., BORGATTI, M., MILANESI, G. & BARBANTI-BRODANO, G. (1982). Transformation of human embryonic fibroblasts by BK virus, BK virus DNA and a subgenomic BK virus DNA fragment. *Journal of General Virology* **63**, 393–403.

LAIMINS, L. A., KHOURY, G., GORMAN, C., HOWARD, B. & GRUSS, P. (1982). Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. *Proceedings of the National Academy of Sciences, U.S.A.* **79**, 6453–6457.

LAND, H., PARADA, L. & WEINBERG, R. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature, London* **304**, 596–602.

LUSKY, M. & BOTCHAN, M. (1981). Inhibition of SV40 replication in simian cells by specific pBR322 DNA sequences. *Nature, London* **293**, 79–81.

MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: A Laboratory Manual.* New York: Cold Spring Harbor Laboratory.

MILANESI, G., BARBANTI-BRODANO, G., NEGRINI, M., LEE, D., CORALLINI, A., CAPUTO, A., GROSSI, M. P. & RICCIARDI, R. P. (1984). BK virus-plasmid expression vector that persists episomally in human cells and shuttles into *Escherichia coli*. *Molecular and Cellular Biology* **4**, 1551–1560.

NEWBOLD, R. F. & OVERELL, R. W. (1983). Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-*ras* oncogene. *Nature, London* **304**, 648–651.

OSBORN, M. & WEBER, K. (1983). Biology of disease. Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. *Laboratory Investigation* **48**, 372–394.

PAGNANI, M., NEGRINI, M., RESCHIGLIAN, P., CORALLINI, A., BALBONI, P. G., SCHERNECK, S., MACINO, G., MILANESI, G. & BARBANTI-BRODANO, G. (1986). Molecular and biological properties of BK virus-IR, a BK virus variant isolated from a human tumor. *Journal of Virology* **59**, 500–505.

PAGNANI, M., CORALLINI, A., CAPUTO, A., ALTAVILLA, G., SELVATICI, R., CATOZZI, L., POSSATI, L. & BARBANTI-BRODANO, G. (1988). Cooperation in cell transformation between BK virus and the human c-Harvey-*ras* oncogene. *International Journal of Cancer* (in press).

PURCHIO, A. F. & FAREED, G. C. (1979). Transformation of human embryonic kidney cells by human papovavirus BK. *Journal of Virology* **29**, 763–769.

ROSENTHAL, N., KRESS, M., GRUSS, P. & KHOURY, G. (1983). BK viral enhancer element and a human cellular homolog. *Science* **222**, 749–755.

RULEY, H. E. (1983). Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature, London* **304**, 602–606.

SANTOS, E., TRONICK, S. R., AARONSON, S. A., PULCIANI, S. & BARBACID, M. (1982). T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB-C and Harvey-MSV transforming genes. *Nature, London* **298**, 343–347.

SEIF, I., KHOURY, G. & DHAR, R. (1979). The genome of the human papovavirus BKV. *Cell* **18**, 963–977.

SLAMON, D. J., DE KERNION, J. B., VERMA, I. M. & CLINE, M. J. (1984). Expression of cellular oncogenes in human malignancies. *Science* **224**, 256–262.

SPANDIDOS, D. A. & WILKIE, N. M. (1984). Malignant transformation of early passage rodent cells by a single mutated human oncogene. *Nature, London* **310**, 469–475.

TAKEMOTO, K. K., LINKE, H., MIYAMURA, T. & FAREED, G. C. (1979). Persistent BK papovavirus infection of transformed human fetal brain cells. *Journal of Virology* **29**, 1177–1185.

TEGTMEYER, P. (1980). Genetics of SV40 and polyoma virus. In *DNA Tumor Viruses: Molecular Biology of Tumor Viruses*, part 2, pp. 297–337. Edited by J. Tooze. New York: Cold Spring Harbor Laboratory.

THOR, A., HAND, H. P., WUNDERLICH, D., CARUSO, A., MURARO, R. & SCHLOM, J. (1984). Monoclonal antibodies define differential *ras* gene expression in malignant and benign colonic diseases. *Nature, London* **311**, 562–565.

VARMUS, H. E. (1984). The molecular genetics of cellular oncogenes. *Annual Review of Genetics* **18**, 553–612.

VASAVADA, R., EAGER, K. B., BARBANTI-BRODANO, G., CAPUTO, A. & RICCIARDI, R. P. (1986). Adenovirus type 12 early region 1A proteins repress class I HLA expression in transformed human cells. *Proceedings of the National Academy of Sciences, U.S.A.* **83**, 5257–5261.

WULF, E., DEBOBEN, A., BAUTZ, F. A., FAULSTICH, H. & WIELAND, T. (1979). Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proceedings of the National Academy of Sciences, U.S.A.* **76**, 4498–4502.

YASWEN, P., GOYETTE, M., SHANK, P. R. & NELSON, F. (1985). Expression of c-Ki-*ras*, c-Ha-*ras*, and c-*myc* in specific cell types during hepatocarcinogenesis. *Molecular and Cellular Biology* **5**, 780–786.

YOSHIIKE, K. & TAKEMOTO, K. K. (1986). Studies with BK virus and monkey lymphotropic papovavirus. In *The Polyomaviruses: The Papovaviridae*, vol. 1, pp. 295–326. Edited by N. P. Salzman. New York & London: Plenum Press.

(Received 30 March 1988)