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Hamster cell line suitable for transfection assay of transforming genes

(focus assay/recipient cells/cellular oncogenes/thyroid carcinoma/Ki-*ras*)

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ABSTRACT **We established a subclone, SHOK, from the GHE-L cell line, an immortal line derived from a primary culture of Syrian hamster embryo cells, as a recipient cell line useful for the detection of oncogenes by transfection. SHOK cells were almost as susceptible as NIH 3T3 cells to focus formation by many oncogenes, including v-*raf*, v-Ha-*ras*, v-Ki-*ras*, or activated c-Ha-*ras*. The susceptibility of SHOK to focus formation was higher than that of NIH 3T3 for v-*mos* but was lower for v-*fps*, v-*fgr*, v-*src*, v-*sis*, and v-*abl*. When DNAs extracted from 27 human and murine tumors were tested for focus formation, 5 DNAs were positive in NIH 3T3 cells, whereas 9 were positive in SHOK cells at the primary transfection. Using SHOK cells as recipients of tumor cellular DNA, we isolated another oncogene and a c-Ki-*ras2* gene mutated at codon 146 that were difficult to detect in NIH 3T3 cells. SHOK cells have a low rate of spontaneous transformation, produce easily distinguishable foci, and maintain a stable karyotype in transformed cells. In addition to being useful for the screening of human tumor DNAs, SHOK cells will be useful for the isolation of oncogenes from murine tumors because of their hamster origin.**

Development of transfection methods for DNA of high molecular weight into mammalian cells (1) has made possible the isolation of many cellular functional genes, including activated cellular oncogenes (2, 3). As recipient cells for assays of cellular-transforming genes, NIH 3T3 cells have been widely used, mainly because of their high susceptibility to transformation. In general, however, the expression of the transfected genes is affected by genetic and physiological conditions of the recipient cells. For example, with viral oncogenes v-*myc* or v-*myb*, it is likely that the transforming activities of some cellular genes are unexpressed or suppressed in NIH 3T3 cells and that their expression becomes apparent in other cells with different regulations of gene expression or different metabolic functions. A recipient cell line that could be used to detect such transforming genes would be of use.

The role of the activation of cellular oncogenes in carcinogenesis has been analyzed by experimental animal models of carcinogenesis; most of such models have used mice. However, it has been difficult to identify transforming genes in mice tumor cells when not focusing on oncogenes already characterized because species-specific sequences that are dispersed and much repeated in mammalian genomes (4, 5) were used as molecular markers to distinguish transfected DNA from host DNA. Thus, the development of recipient cell lines of species other than mice should help elucidate the activation of protooncogenes in mouse carcinogenesis. Though several rodent cell lines other than NIH 3T3 are used as recipient cells for detecting cellular oncogenes, these lines had few advantages over NIH 3T3 cell use (6, 7).

Here, we report the development of a hamster cell line for detecting cellular oncogenes.

MATERIALS AND METHODS

Cell Lines and Media. The GHE-L cell line was established with a culture of high cell density from a primary culture of Syrian hamster embryo after 50 passages (F.S., unpublished work) at a density of 2×10^6 cells per 50 cm^2 every 8 days. The SHOK cell line was subcloned from the GHE-L cells. These cells were maintained at low cell densities in Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented with 10% fetal calf serum (FCS). For the focus assay, DMEM supplemented with 3% FCS was used. A subclone from the NIH 3T3 cell line was selected for its flat morphology and low incidence of spontaneous transformation. NIH 3T3 cells were maintained in DMEM supplemented with 10% calf serum. For focus assay of NIH 3T3 cells, DMEM supplemented with 5% calf serum was used.

Preparation and Sources of DNAs. Cellular DNAs of high molecular weight (8) and plasmid and bacteriophage DNAs (9) were extracted by the described method.

Cloned DNAs were provided, directly or through the Japanese Cancer Research Source Bank, by K. Shimizu (Kyushu University, Fukuoka, Japan; pT22), I. M. Verma (The Salk Institute; pMSV-1L), N. Tsuchida (Tokyo Medical and Dental University; pSV2-v-mos and p4E), U. R. Rapp (National Cancer Institute; 3611E-H), E. M. Scolnick (National Cancer Institute; pH1), J. M. Bishop (University of California, San Francisco; pSRA-2 and pAE-11), A. Srinivasan (National Cancer Institute; pAB6.1), R. C. Gallo (National Cancer Institute; pc60), R. A. Weinberg (Massachusetts Institute of Technology; pSVc-myc and pSW11-1), H. Hanafusa (The Rockefeller University; λ-FSV-2), M. A. Baluda (UCLA School of Medicine and Molecular Biology Institute; λ11A1-1), P. Berg (Stanford University; pSV2-neo), C. W. Schmid (University of California, Davis; BLUR-8), and H. Yamagishi (Kyoto University; p014; see ref. 5). Plasmid pSV2neo-fgr was our own (10).

Cell lines that contain activated cellular *ras* genes (11–13) were provided by R. C. Gallo (HL-60), K. Shimizu (T24), and Y. Taya (National Cancer Center Research Institute, Tokyo; NIH 3T3-Lu65).

We tested 27 tumor DNAs, 10 from cell lines and 17 from tumor tissues, for their transforming activities toward SHOK and NIH 3T3 cells. The human thyroid carcinoma cell line,

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Abbreviations: DMEM, Dulbecco's modified Eagle's minimum essential medium; FCS, fetal calf serum.
§Deceased September 21, 1988.

Sento, was from S. Noguchi (The Center for Adult Diseases, Osaka), and four human colon carcinoma cell lines, CC-06, -07, -011, and -014, were from N. Kikkawa (National Osaka Hospital). One tumorigenic human fibroblast cell line, HuT-14 (14), and four human thyroid carcinoma cell lines, TCO-1, -3, -4, and -5, were our own. Twelve specimens of human colon carcinoma, CT-1, -2, -3, -4, -6, -7, -8, -9, -10, -11, -12, and -13, were from N. Kikkawa, and five murine tumors, N5-2057, N5-BMX-2305, -2596, -2710, and -3013 were from T. Nomura (Osaka University).

Transfection Assay. Transfection was done by precipitation with calcium phosphate (1). One nanogram to 1 μg of plasmid or 100 ng to 3 μg of bacteriophage DNA with 10 μg of carrier NIH 3T3 DNA was transfected into 2×10^5 SHOK or NIH 3T3 cells that had been seeded onto 6-cm Petri dishes the previous day. Media were refreshed two or three times a week, and transformed foci were scored after 10–14 days. For focus assays of cellular DNAs, 5×10^5 cells were seeded onto 10-cm Petri dishes, and 30–33 μg of DNAs was transfected. After 2–3 weeks, transformed foci were scored, and transformed cells were cloned from the foci.

Southern Blot Analysis. Cellular DNAs were digested with restriction endonucleases (Takara Shuzo, Kyoto, Japan) under the conditions recommended by the supplier. Ten micrograms of digested DNAs were fractionated by electrophoresis in 0.7% agarose gels and blotted onto nitrocellulose or nylon filters by the method of Southern (15). The filters were hybridized with probes labeled with [α-^{32}P]dCTP by a multiprimer DNA labeling kit (Amersham), washed, and used to expose Kodak films as described elsewhere (9).

Characterization of SHOK and Transformed Cells. To measure the doubling time, saturation density, and serum dependency, cell growth in 10%, 3%, 1%, and 0% FCS was observed. For dependency on anchorage, colony formation in soft agar was examined. Ten thousand SHOK cells or 1×10^3 transformed cells was seeded into 0.25% agar containing DMEM/20% FCS, and colonies with diameters of 100 μm or more were scored after 3-week culture. For tumorigenicity, 5×10^6 SHOK cells or 1×10^6 transformed cells was injected s.c. into Syrian hamsters 4–6 weeks old. Tumor formation was observed for 20 weeks.

Molecular Cloning and cDNA Expression Plasmids. To clone the transforming gene from the cells transformed with TCO-4 DNA, we constructed bacteriophage λ2001 (16) libraries by inserting *Eco*RI or *Mbo* I partial digests and also constructed a cosmid pHSG274 (17) library by inserting *Mbo* I partial digests of DNA from tertiary transformants. These libraries were screened with human repetitive *Alu* sequence as probe (4). To isolate the cDNA of the transforming gene, we extracted and purified mRNA from the same transformants as described (9). We constructed a cDNA library from the mRNA using a cDNA cloning kit (Amersham) and screened the cDNA library with some genomic fragments of the transforming gene as probes.

To clone the activated Ki-*ras* gene from HuT-14 DNA, we constructed λ2001 libraries by inserting *Eco*RI or *Hin*dIII partial digests of DNA from secondary transformants and screened the libraries with *Alu* sequence as probe. We also screened a cDNA library from the secondary transformants with probes specific for human Ki-*ras* gene.

To construct cDNA expression vectors, we removed the 3-kilobase pair (kbp) *Kpn* I fragment specific for v-*fgr* from the plasmid pSV2neo-fgr and inserted cDNAs into the *Kpn* I site between the long terminal repeats of Gardner–Rasheed feline sarcoma virus.

RESULTS

Subcloning of the Hamster Cell Line SHOK from GHE-L Cells. GHE-L cells formed transformed foci by transfection with pT22 (T24-c-Ha-*ras*1 gene; F.S., unpublished work). The transforming efficiency of GHE-L cells was lower than that of NIH 3T3 cells, and it was sometimes difficult to distinguish authentic foci from pseudofoci in GHE-L cells because of their heterogeneity. To use GHE-L cells as a recipient for the detection of cellular oncogenes, we subcloned the cells and found one subclone with transforming efficiency by pT22 and transfection efficiency of G418 resistance gene almost equal to those of NIH 3T3 cells (data not shown). We named the subclone SHOK for Syrian hamster cells Osaka–Kanazawa and examined its use as a recipient for oncogenes.

Comparison of Focus-Forming Efficiencies Between SHOK and NIH 3T3 Cells. Table 1 shows the results of transfection assays of various oncogenes. Oncogenes could be classified into four groups by means of focus-forming abilities with SHOK and NIH 3T3 cells. SHOK cells were more efficiently transformed by plasmid pMSV-1L and pSV2-v-mos than were NIH 3T3 cells. These are v-*mos* genes under the control of different promoters, so the SHOK cells seemed to be more sensitive to transformation by the v-*mos* gene than were the NIH 3T3 cells. However, SHOK cells were transformed at lower efficiency by v-*fgr*, v-*src*, v-*sis*, v-*abl*, and v-*fps*. Both cells had similar transforming efficiencies (<3-fold) by v-*raf*, v-Ki-*ras*, v-Ha-*ras*, and activated c-Ha-*ras*, and formed no foci with c-*myc*, proviral DNA of avian erythroblastosis virus (pAE-11), or proviral DNA of avian myeloblastosis virus (λ11A1-1).

Detection and Identification of Transforming Genes Derived from Tumor DNAs. We tested the 27 tumor DNAs listed above for their focus-forming activities in SHOK and NIH 3T3 cells. DNAs extracted from cells transformed by tumor DNAs were analyzed by Southern blot hybridization with the repetitive sequences as probes (see Fig. 2 *A–C*) and put through the secondary transfection assays. Transformant DNAs derived from the 11 tumor DNAs listed in Table 2, except for N5-BMX-2596, contained human or murine repetitive sequences and formed foci in the secondary transfection assays. A few transformants did not contain repetitive sequences and did not form foci at the secondary transfection,

Table 1. Focus-forming efficiencies of various oncogene-bearing DNAs in SHOK and NIH 3T3 cells

DNA	Oncogene	Focus-forming units per fmol* SHOK	NIH 3T3
Plasmid			
pMSV-1L	v-*mos*	13 ± 5.3	0.29 ± 0.26
pSV2-v-mos	v-*mos*	27 ± 13	4.9 ± 2.7
3611E-H	v-*raf*	20 ± 4.5	17 ± 1.7
pT22	c-Ha-*ras*	54 ± 21	50 ± 34
p4E	v-Ki-*ras*	26 ± 23	48 ± 19
pH1	v-Ha-*ras*	21 ± 6.3	45 ± 2.2
pSV2neo-fgr	v-*fgr*	0.26 ± 0.12	2.6 ± 0.35
pSRA-2	v-*src*	0.12 ± 0.052	2.6 ± 0.24
pc60	v-*sis*	<0.003	0.045 ± 0.013
pAB6.1	v-*abl*	<0.003	0.17 ± 0.041
pAE-11	v-*erb*A,B	<0.003	<0.003
pSVc-myc	c-*myc*	<0.003	<0.003
Bacteriophage			
λ-FSV-2	v-*fps*	0.64 ± 0.64	5.1 ± 1.4
λ11A1-1	v-*myb*	<0.003	<0.003
Cellular			
HL-60	N-*ras*	1.1 ± 0.61†	0.41 ± 0.11†
T24	c-Ha-*ras*	2.3 ± 0.92†	1.4 ± 0.29†
NIH 3T3-Lu65	c-Ki-*ras*	0.53 ± 0.37†	0.97 ± 0.19†

*Mean ± SD of three to eight dishes.
†Focus-forming units per μg of DNA.

Table 2. Transfection assay of tumor DNAs with SHOK and NIH 3T3 cells

DNA	Primary assay		Secondary assay*	
	SHOK	NIH 3T3	SHOK	NIH 3T3
Human thyroid carcinoma cell line				
TCO-4	1/6	0/6	35/4	1/4
Human fibroblast tumorigenic cell line				
HuT-14	29/20	0/20	17/4	0/4
Human colon carcinoma cell line				
CC-014	5/6	0/6	50/2	9/2
Human colon carcinoma specimen				
CT-6	0/6	1/6	110/2	7/2
CT-8	0/6	3/6	90/2	74/2
CT-10	8/6	1/6	36/2	9/2
CT-12	1/6	0/3	4/2	0/5
CT-13	1/6	0/3	226/2	7/2
Murine tumor specimen				
N5-2057	7/13	0/11	129/2	111/2
N5-BMX-2596	0/17	3/9	0/15	0/13
N5-BMX-2710	1/22	1/9	2/3	8/14
N5-BMX-3013	1/10	0/7	43/3	6/3

Thirty to 33 μg of cellular DNA was transfected into 5×10^5 cells per 10-cm dish. After 3 weeks, transformed foci were scored, and transformed cells were cloned from the foci. DNA from the transformed cells was examined for repetitive sequences and used for the transfection assay of the next cycle. Among the 27 tumor DNAs tested, the 12 listed in the table had focus-forming activities toward SHOK cells, NIH 3T3 cells, or both. Results are expressed as number of foci per number of dishes tested.

*For CT-6, CT-8, and N5-BMX-2596 specimens, the secondary transfection assays were done with NIH 3T3 focus-derived DNAs. In other cases, SHOK focus-derived DNAs were used for the secondary assays.

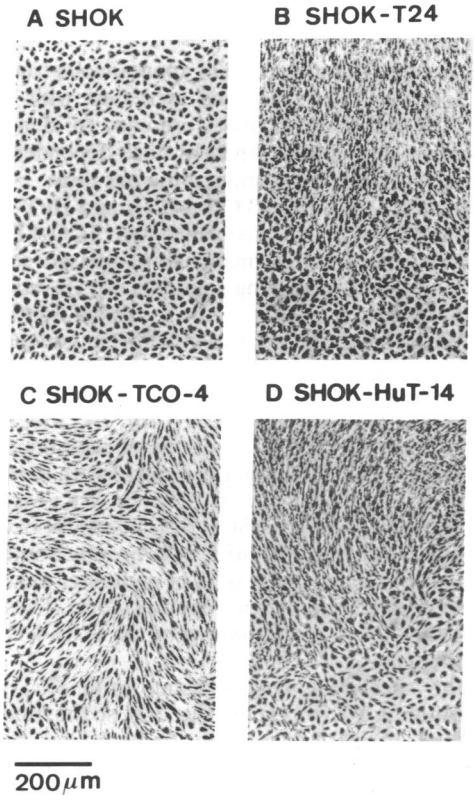

FIG. 1. Phase-contrast photomicrographs of SHOK cells (*A*) and the transformed foci by DNAs of T24 (*B*), TCO-4 (*C*), and HuT-14 (*D*) cells. (Bar = 200 μm.)

so they seemed to be derived from spontaneous transformation. We could not examine for repetitive sequences in the transformants derived from the DNA of N5-BMX-2596, which formed foci only in NIH 3T3 cells, because of its mouse origin. Because the focus-forming activity of N5-BMX-2596 was weak, we could not confirm the authenticity of the transformants.

Of the 27 tumor DNAs tested, 5 (19%) formed foci in NIH 3T3 cells, and 9 (33%) formed foci in SHOK cells (Table 2 and Fig. 1). Among the 22 DNAs that did not form foci on NIH 3T3 cells, 7 formed foci in SHOK cells. At the secondary transfection assays, the DNAs of primary transformants of SHOK derived from HuT-14 and CT-12 had focus-forming activities only in SHOK cells, and the DNAs of the transformants from the other 9 tumor DNAs had focus-forming activity in both cells. However, SHOK cells formed foci more efficiently with the DNAs that were negative in NIH 3T3 at the primary transfection than did NIH 3T3 cells. Although DNA of the primary transformant of SHOK derived from TCO-4 DNA had weak focus-forming activity in NIH 3T3 cells, the appearance of foci was later in NIH 3T3 cells than in SHOK cells and the transformants of NIH 3T3 could not form colonies with diameters >300 μm in soft agar.

Southern blot analysis showed that most transforming genes of the tumor DNAs were members of the *ras* gene family (HuT-14, CC-014, CT-6, CT-8, CT-10, CT-13, and N5-2057), but the transforming genes of TCO-4, CT-12, and N5-BMX-3013 DNAs were not *ras*-family members. With N5-2057 DNA, one transformant contained an activated *mos* gene, whereas the other transformants contained an activated Ki-*ras* gene, and the DNAs of both transformants had focus-forming activities in SHOK and NIH 3T3 cells.

Properties of SHOK Cells and the Transformed Cells. SHOK cells and the cells transformed by DNAs of T24, HuT-14, and TCO-4 were examined for properties (Table 3). Plating efficiencies of SHOK and the transformed cells were ≈70%. The transformed cells had shorter doubling times, higher saturation densities, and less need for serum than the untransformed SHOK cells. The transformed cells could grow in 1% serum, but untransformed cells could not. No cells tested could grow without serum. SHOK cells were anchorage-dependent and nontumorigenic in hamsters. However, the transformed cells formed colonies in soft agar and rapidly growing anaplastic tumors in hamsters after a latent period of 2–4 weeks. SHOK cells were stable in karyotype even after transformation by oncogenes: monosomy of chro-

Table 3. Properties of SHOK cells and the transformed cells

Strain	Chromosome number	Plating efficiency, %	Doubling time, hr		Saturation density, no. $\times 10^5$ cells/cm^2		Colony-forming efficiency in soft agar, %	Tumorigenicity in hamster*
			10% FCS	3% FCS	10% FCS	3% FCS		
SHOK	45	68	14.9	18.7	1.3	0.58	<0.01	0/10
SHOK-T24	45	76	8.2	9.4	4.3	3.9	30.7	5/5
SHOK-TCO-4	44	71	12.1	17.0	3.3	1.7	12.8	17/18
SHOK-HuT-14	45	68	12.0	13.8	3.6	1.8	39.6	5/5

*Number of tumors per number of animals tested.

mosome 5, trisomy of chromosome 11, and a marker chromosome ($14q^+$).

In SHOK cells, the incidence of spontaneous transformation was low (0.07 per dish) and increased with repeated passages. The transformed foci were easy to identify, and transformation was almost linearly dose-dependent within the range of 5–100 focus-forming units per dish.

Transforming Genes from TCO-4 DNA and HuT-14 DNA. DNAs of the SHOK cells transformed with TCO-4 DNA and DNAs of the tertiary transformants contained *Alu* sequences (Fig. 2*A*), and the transforming gene was not Ha-*ras*, Ki-*ras*, N-*ras*, *raf*, or *mos*, according to the results of Southern blot analysis. We isolated the genomic transforming sequence and its cDNA from the tertiary transformants. The physical map of the transforming sequence was not identical to that of Ha-*ras*, Ki-*ras*, N-*ras*, *mel*, *dbl*, *ret*, *raf*, *trk*, *hst*, *lca*, *met*, *mcf-1*, *mcf-2*, or the thyroid cancer-associated oncogene (3, 18, 19), and the cDNA did not hybridize with v-*src*, v-*fgr*, v-*fps*, v-*fes*, v-*ros*, v-*erbB*, v-*raf*, v-Ha-*ras*, v-Ki-*ras*, v-*erbA*, c-*erbB-2*, B-*lym-1*, v-*yes*, v-*abl*, v-*fms*, v-*mos*, N-*ras*, v-*myc*, v-*fos*, v-*myb*, v-*rel*, v-*sis*, N-*myc*, or L-*myc* by Southern blot analysis. According to the results of computer analysis, the cDNA also lacked homology to reported genes. These results suggested that the transforming gene of TCO-4 DNA was another oncogene.

Even the secondary transformants with HuT-14 DNA contained human c-Ki-*ras* gene (Fig. 2*D*), so the transforming gene of HuT-14 DNA was likely to be Ki-*ras* gene. This finding was of interest because NIH 3T3 cells formed no foci by transfection with HuT-14 DNA in repeated experiments, and no tumors positive for *Alu* sequence were found in tumorigenicity assays with NIH 3T3 cells used as recipients (20). To study the mechanism involved in the activation of the Ki-*ras* gene, we analyzed its genomic and cDNA sequences and found a point mutation at codon 146 that substituted the residue proline for alanine. Mutation of this position of *ras* genes has not been reported in NIH 3T3 assays.

Focus-Forming Activities of Transforming Genes. As human c-Ki-*ras2* gene is ≈40 kbp long and the transforming gene from TCO-4 DNA was >30 kbp long, it was difficult to isolate biologically active genomic clones of these oncogenes. Thus, to investigate the transforming activities of transforming genes from HuT-14 and TCO-4 DNA, we constructed cDNA expression plasmids (Table 4). pJJ26, a cDNA expression plasmid of the transforming gene from TCO-4 and pLTRKras(Pro146), a cDNA expression plasmid of c-Ki-*ras2* gene from HuT-14, had focus-forming activities both in SHOK and NIH 3T3 cells. In NIH 3T3 cells, pLTRKras(Pro146) had ≈5-fold lower focus-forming activity than pLTRKras(Val12), an expression plasmid of human c-Ki-*ras2* mutated at codon 12. However, both Ki-*ras* expression plasmids had similar focus-forming activities in SHOK cells.

Table 4. Focus-forming activities of cloned transforming genes

DNA	Focus-forming units per pmol	
	SHOK	NIH 3T3
pLTRKras(normal)	<3	<3
pLTRKras(Val12)	360 ± 55	2300 ± 450
pLTRKras(Pro146)	260 ± 21	420 ± 45
pJJ26	980 ± 2	1500 ± 160

pLTRKras is an expression vector of human Ki-*ras* cDNA; Kras(normal), normal Ki-*ras* cDNA; Kras(Val12), Ki-*ras* cDNA from SW480; Kras(Pro146), Ki-*ras* cDNA from HuT-14. pJJ26 is a cDNA expression plasmid of the transforming gene from TCO-4. Data shown are mean ± SD of three to six dishes.

DISCUSSION

The NIH 3T3 cell line has been much used as recipient cells for the detection of oncogenes. Some rodent cells are transformed by transfection with oncogenes, but only a few—Rat-2 (6) and C3H10T½ (7)—have been employed as recipient cells for the detection of cellular oncogenes. Most of these lines proved lower in transforming efficiency than NIH 3T3 cell line (6) and more difficult to use. C3H10T½ cells are transformed efficiently (7), but they do not detect any oncogenes other than those that transform NIH 3T3 cells. To our knowledge, there have been no reports on the isolation of additional cellular oncogenes by use of cell lines other than NIH 3T3.

SHOK cells seemed to satisfy many conditions required for recipient cells. They were susceptible to transfection with exogenous genes and to transformation by various oncogenes. Transformed foci were easily distinguished from untransformed cells, and the frequency of spontaneous transformation was low. Transformations by oncogenes were dose-dependent and reproducible. These cells were easy to

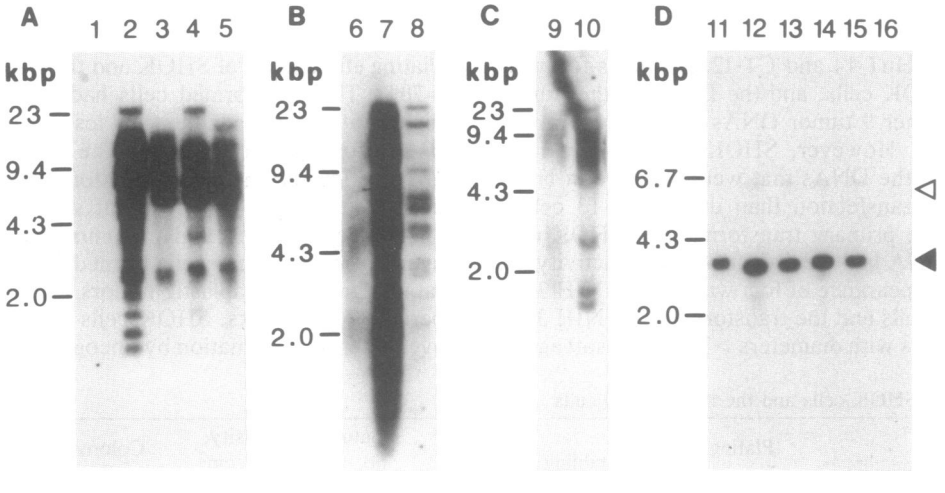

FIG. 2. Detection of human repetitive *Alu* sequence (*A* and *B*), murine repetitive sequence (*C*), and human Ki-*ras* gene (*D*) in SHOK cells transformed with tumor DNAs. Ten micrograms of each cellular DNA was digested with the restriction endonuclease *Eco*RI, fractionated by gel electrophoresis, and analyzed by Southern blot hybridization with BLUR-8 (*A* and *B*), p014 (*C*), and the 280-bp *Dra* I fragment containing the second exon of human c-Ki-*ras2* gene (*D*) as probes. Lanes: 1, 6, 9, and 16, SHOK cells; 2, primary transformant by TCO-4 DNA; 3 and 4, secondary transformants by TCO-4 DNA; 5, tertiary transformant by TCO-4 DNA; 7, 8, 12, and 13, primary transformants by HuT-14 DNA; 10, primary transformant by N5-2057; 11, HuT-14 cells; 14 and 15, secondary transformants by HuT-14 DNA. In *D*, solid and open triangles indicate human and hamster bands, respectively.

handle in culture because of high plating efficiency, short doubling time, and no requirement for a specific medium. Unlike NIH 3T3 cells, SHOK cells had the advantage of karyotype stability, which would facilitate analysis of the functions of exogenous genes. Compared with NIH 3T3 cells, SHOK cells had different sensitivity to transformation by various oncogenes and were particularly susceptible to transformation by the v-*mos* gene. SHOK cells were as useful as NIH 3T3 cells for detecting cellular *ras* genes, which are frequently activated in tumors.

Transforming activities were detected in 44% of the tumor-cellular DNAs tested by use of SHOK cells in combination with NIH 3T3 cells. Seven cellular DNAs with focus formation were detected in SHOK cells but not in NIH 3T3 cells. Although most of them produced foci in NIH 3T3 cells, as well as in SHOK cells, at the secondary transfection, their focus-forming efficiencies were lower in NIH 3T3 than in SHOK cells. The DNA from HuT-14 cells produced no foci with NIH 3T3 cells and was found to contain a c-Ki-*ras2* gene mutated at codon 146. The DNA from TCO-4 cells had weak focus-forming activity in NIH 3T3 cells at the secondary transfection and was found to contain another oncogene. Under the control of a strong promoter, the cDNA of transforming gene from HuT-14 or TCO-4 had focus-forming activity in NIH 3T3 cells as well as in SHOK cells. However, the results of focus-forming assays of cellular DNAs suggested that, using SHOK cells as recipient cells, we could detect some transforming genes difficult to detect in NIH 3T3 cells.

Transfected murine sequences are more easily detected in SHOK cells than in NIH 3T3 cells, so SHOK cells will be of use for detecting cellular oncogenes from murine tumors.

Our results suggest that the SHOK cell line will be useful for studies of oncogenes and the cellular factors affecting oncogene functions. Of course, many oncogenes will escape detection by either SHOK or NIH 3T3 cells, and screening systems must be devised for such oncogenes.

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