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Abstract

Trial of Rous sarcoma virus (RSV) induction by cell fusion with chick embryo cells (CEC) and wing web test from so-called RSV-transformed human cells, KC and RSb cells, was unsuccessful. The loss of RSV inducibility was also confirmed by DNA transfection method. Southern blot and northern blot hybridization of DNA and RNA from those cells with the v-src probe revealed that the v-src genes in those cells were defective and not expressed. On the other hand, the v-src gene in RSV-transformed mouse and rat cells was complete and transforming virus was inducible from them.

KEYWORDS: src gene, human cells, RSV induction, Southern blot hybridization, northern blot hybridization

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V-*src* Genes in Two Cell Lines of So-Called RSV-Transformed Human Cells are Defective and Inactive

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Trial of Rous sarcoma virus (RSV) induction by cell fusion with chick embryo cells (CEC) and wing web test from so-called RSV- transformed human cells, KC and RSb cells, was unsuccessful. The loss of RSV inducibility was also confirmed by DNA transfection method. Southern blot and northern blot hybridization of DNA and RNA from those cells with the v-*src* probe revealed that the v-*src* genes in those cells were defective and not expressed. On the other hand, the v-*src* gene in RSV-transformed mouse and rat cells was complete and transforming virus was inducible from them.

Key words : ***src* gene, human cells, RSV induction, Southern blot hybridization, northern blot hybridization**

Rous sarcoma virus (RSV) transforms mammalian cells, although the transforming efficiency is low compared to chick embryo cells (CEC). Regarding human cells, it has been reported that KC (1) and RSb cells (2) have been transformed by RSV infection. On the other hand, it has also been demonstrated that human fibroblasts could not be transformed by the v-*src* gene, although the gene was vigorously expressed in these cells (3).

In retroviruses, the whole or a part of the viral genome is integrated in a stable form into the host chromosomal DNA after infection and transformation. In some cases, however, rearrangement or excision of the viral genome may occur. It is believed that the *src* gene and the long terminal repeats (LTRs) of RSV are sufficient for the maintenance of transformation (4).

The present study was concerned with demonstrating the lack of RSV inducibility from human KC and RSb cells, and the defectiveness in structure and function of v-*src* gene in these cells.

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Materials and Methods

Cells and DNA probe. The specific pathogen-free C/O CEC were obtained from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University, Kanonij, Japan. CEC were transnformed by the Schmidt-Ruppin strain of RSV, CEC/RSV, in this laboratory. Human glioma cell line, 118MG and cells transformed by the Engelbreth-Holm strain of RSV, KC (1), was obtained from Dr. J. Ponten, Uppsala, Sweden in 1975. Human embryonic cells doubly transformed by

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RSV and SV40, RSb (2), and mouse ascites cells transformed by the Schmidt-Ruppin strain of RSV, SR-C3H/He (5), were obtained from Dr. T. Oda, Okayama, Japan in 1976. Rat cells transformed by the Prague strain of RSV [XC (6)], normal rat kidney cells transformed by the Bratislava strain of RSV [NRK/B77], mouse A31 cell line and A31 cells transformed by the Bratislava strain of RSV [A31/B77] have been kept in this laboratory.

The v-*src*-LTR probe, p*src*2, *Eco*R I fragment of the Schmidt-Ruppin strain of RSV containing the *src* gene and a part of LTR of 2.95 kb (7), was obtained from Dr. J. Hillova, Villejuif, France.

Cell fusion and wing web test. Mammalian cells (5×10^6 cells) transformed by RSV were fused with C/O CEC (1×10^7 cells) by the method using $80 \mu g/ml$ phytohemaggulutinin (PHA-P, Difco Lab., Michigan, USA) and 50 % polyethylene glycol (PEG 1000, Ishizu Co., Japan) (8). The culture supernatants of these fused cells were inoculated onto C/O CEC to test the presence of rescued RSV. Mammalian cells(1×10^7 cells) transformed by RSV were inoculated into wing webs of one-day-old chicks and observed for tumor formation. The tumors were homogenized, sterilized by filtration and inoculated onto C/O CEC to test the presence of RSV.

Extraction and digestion of DNA. Extraction and purification of high molecular weight DNA from culture cells were performed as described previously (9). Digestion of DNA by endonuclease *Eco*R I (Boehringer Mannheim Co., Mannheim, W. Germany) was performed according to the methods indicated by the manufacturer.

Transfection. Transfection of C/O CEC was performed by the calcium method combined with dimethyl sulfoxide treatment as described previously (10). The CEC cultures were maintained for 2 weeks and the appearance of transformed foci was monitored. To be sure that RSV was rescued from the transfected CEC, the culture supernatants were inoculated onto C/O CEC for transformation.

DNA labeling and Southern blot hybridization. The DNA probe p*src*2 was labeled with digoxigenin-deoxyuridine triphosphate using the kit prepared by Boehringer Mannheim, Mannheim, W, Germany. The electrophoresis of *Eco*R I-digested $10 \mu g$ of DNA was done in 0.8 % agarose gel in TEA-NaCl buffer (50 mM Tris-HCl (pH 8.05) containing 20 mM Sodium acetate, 2 mM Na_2EDTA and 18 mM NaCl). After gel electrophoresis, DNA was transferred to Nytran membrane (Schleicher and Schuell, Passel, W. Germany) by Southern blotting (11). After hybridization under stringent conditions, the detection of the hybrids was performed by enzyme-linked immunoassay according to the manual prepared by the manufacturer of the kit.

Northern blot hybridization. Total RNA of KC, RSb and XC cells was extracted by the guanidinium-hot phenol method (12). Hybridization of denatured RNA was perfomed according to the manual described previously (13). Twenty micrograms of total RNA from each cell were denatured, electrophoresed in 1 % agarose-formaldehyde gel and transferred to a membrane filter. The filter was hybridized with ^{32}P-labeled v-*src* probe in solution containing 50 % formamide, $5 \times$ standard saline citrate (SSC), $5 \times$ Denhard's solution (0.1 % Polyvinylpyrolidone, 0.1 % Ficoll and 0.1 % bovine serum albumin) and 1 % sodium dodesyl sulfate (SDS) at 45°C. The filter was then washed three times with $2 \times$ SSC-0.1 % SDS solution at 60°C and exposed on a X-ray film. To control the amount of RNA loading, rehybridization with human beta-actin probe was performed under the same condition, using the same filter.

Results

RSV induction by cell fusion, wing web test and transfection. As shown in Table 1, RSV was not inducible from KC and RSb cells by cell fusion with CEC or by chick wing web tests, but it was inducible from RSV-transformed mouse and rat cells. Likewise, transfection of CEC with the DNA from KC and RSb cells did not induce RSV, but the DNAs from RSV-transformed mouse, rat and chicken cells did (Table 1).

Detection of v-src by Southern blot hybridization. V-*src*-LTR of 2.95 kb was detected in *Eco*R I digested DNA from RSV-transformed CEC, mouse and rat cells (Fig. 1). DNA bands with molecular weight higher than 2.95 kb were also detected in normal and RSV-transformed CEC as well as RSV-transformed rodent cells (Fig. 1), which represent c-*src* and endogenous LTR DNA sequences homologous to the probe. Bands were not detected at the size of 2.95 kb in *Eco*R I digested DNA from KC and RSb cells, or in 118MG cells, the parent cells of KC (Fig. 1). Only faint bands of DNA molecules larger than 2.95 kb were detected in the DNA from KC and

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RSb, but no band was detectable in the DNA from 118MG cells (Fig. 1).

Detection of v-src gene RNA by northern blot hybridization. The m-RNA containing v-*src* was not detected in RNA from KC and RSb cells, but was detected in RNA from rat XC cells transformed by RSV (Fig. 2). In Fig. 2, two independent autoradiograms for v-*src* and beta-actin were photographed in layers and it was shown that the amount of each RNA loaded in this study was about the same because the band-thickness of the beta-actin was almost the same.

Table 1 Induction of Rous sarcoma virus (RSV)

Cell	Induction of RSV[a] by		
	Cell fusion	Wing Web test	Transfection[b]
Human KC	0/10	0/10	0/20
Human RSb	0/10	0/10	0/13
Mouse A31	0/ 5	n.t.	0/ 5
Mouse A31/B77	4/ 5	n.t.	2/ 5
Mouse SR-C3H/He	5/ 5	3/ 3	8/10
Rat NRK/B77	4/ 5	2/ 3	2/10
Rat XC	5/ 5	3/ 3	2/ 2
CEC/RSV	n.t.	n.t.	2/ 2

a: No. of positive cases for RSV induction/No. of trials; n.t., not tested.
b: The results of RSV induction by transfection of CEC with each cellular DNA was registered.

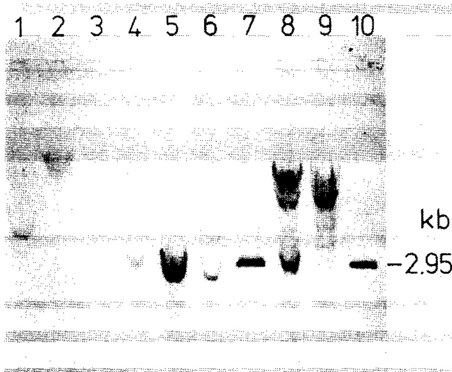

Fig. 1 Detection of the v-*src* gene in cellular DNA. Southern blot hybridization patterns of *Eco*RI digests of each 10 μg of cellular DNA to the v-*src*-LTR probe were shown. Lanes : 1, human RSb cells transformed by RSV and SV 40; 2, human glioma cells transformed by RSV (KC cells); 3, human glioma 118MG cells; 4, A31/B77 cells; 5, SR-C3H/He cells; 6, NRK/B77 cells; 7, XC cells; 8, CEC/RSV; 9, CEC; and 10, v-*src*-LTR DNA of 2.95 kb (5 ng).

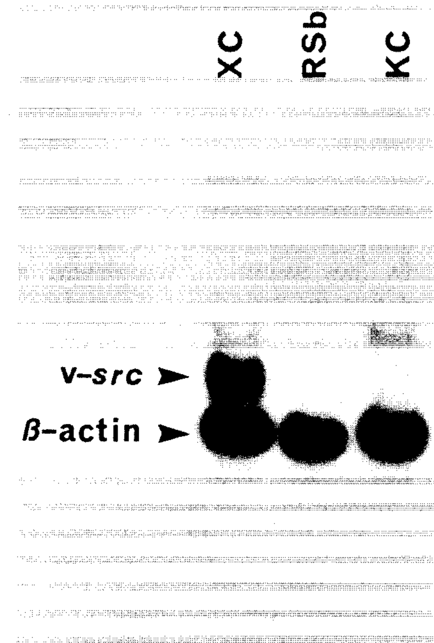

Fig. 2 Detection of the v-*src* RNA by northern blot hybridization. Twenty micrograms of total RNA from each KC, RSb and XC cells were electrophoresed in 1 % agarose-formaldehyde gel, transferred to a membrane filter and hybridized with ^{32}P-labeled v-*src* and beta-actin probes. The v-*src* RNA was detected only in XC cells, while the beta-actin RNA was detected in all samples in about the same amount.

Discussion

The present data demonstrated that RSV was not inducible from human KC and RSb cells which had been reported to be transformed by RSV. Although the lack of RSV inducibility can be caused any defect in RSV genes, we examined the v-*src* gene at first. The results of Southern blot hybridization have revealed that the v-*src* genes in these human cells are defective. Regarding the nature of the defectiveness, deletion or rearrangement of the gene was conceivable.

The v-*src* related cellular sequence, c-*src*, was detected in each family of vertebrates including human (14, 15). In Fig. 1, several bands of c-*src* and endogenous LTR were also detected by v-*src*-LTR probe in DNA from CEC and rodent cells. Although no band was detected this time in DNA from 118MG cells, parent cells of KC cells, DNAs from normal human cells also showed faint bands about the same molecular size of that of KC cells (not shown). Accordingly, it was assumed that the faint band detected in DNA from KC cells represented c-*src* and not modified v-*src* gene. It seemed that no v-*src* gene was present in KC cells. The faint bands detected in the DNA from RSb cells remain to be determined. Further analysis by DNA sequencing may confirm whether they are modified v-*src* or c-*src*.

It was also shown in this study that the v-*src* gene was not expressed, either in KC or RSb cells. From these results, it might be concluded that the v-*src* gene plays at present no role in maintenance of transformation in these so-called RSV-transformed human cells. However, the role of the v-*src* gene at the time of cell transformation is unclear. As it was not possible to examine the earlier stocks of these cells, it remained uncertain when the v-*src* gene changed.

Human KC, RSb and rat XC cells have been used for titration of simian and murine retroviruses in the syncytial plaque counting method (16–19). As those cells were believed to carry the RSV genome, it has been speculated that the v-*src* gene plays a promoting role in syncytia formation by these retroviruses (17, 20). In this study, however, it has been shown that KC and RSb cells tested in this trial now carry a defective v-*src* gene, though they still form syncytia by simian retroviruses (10, 18, 20). Accordingly, it might be concluded that a factor(s) other than v-*src* is responsible for syncytia formation by retroviruses.

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