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XENOGENIZATION OF TUMOR CELLS BY TRANSFECTION WITH PLASMID CONTAINING *env* GENE OF FRIEND LEUKEMIA VIRUS

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A rat hepatocellular carcinoma cell line (cKDH-8 cl-11) showed decreased tumorigenicity after transfection with an envelope gene derived from a Friend leukemia virus (FV-*env* gene). FV-*env* gene product was found by indirect immunofluorescence staining to be expressed on the cell surface of the FV-*env* gene-transfected cells. The FV-*env*-transfected cells (FV-env cKDH-8), however, grew well in X-irradiated immunosuppressed rats, indicating that the reduction in tumorigenicity of the transfected cells is based on immunological reaction in the host. The rats which rejected FV-env cKDH-8 cells showed resistance to rechallenge with the parent cKDH-8 cl-11 tumor cells. These results suggest that the FV-*env* gene product may elicit antitumor immunity against FV-env cKDH-8 cells in a host with a resultant reduction in the tumorigenicity of these cells.

Key words: Xenogenization — Transfection — Friend leukemia virus — *env* gene

We have previously reported that various types of rat tumor cells artificially infected with Friend virus complex (FV) regress spontaneously, since the viral gene products are immunologically recognized as foreign in normal syngeneic rats.[1] A similar result has been reported in the case of endogeneous murine C-type virus.[2] These phenomena have been termed the xenogenization of tumor cells.[1] It has also been reported that tumor regression has been achieved by transfection with an allogeneic class I major histocompatibility complex gene.[3] In this study, we have examined the possibility that the tumor cells transfected with the Friend leukemia virus (F-MuLV) envelope (FV-*env*) gene itself may elicit an enhanced antitumor immunity and may thus regress in syngeneic normal rats.

An inbred strain of WKA/Hok rats, 6 to 10 weeks old, was supplied by the Experimental Animal Institute, Hokkaido University School of Medicine. KDH-8 is a transplantable hepatocellular carcinoma induced by 3′-methyl-4-dimethylaminoazobenzene in a WKA/Hok rat. The cKDH-8 cl-11 is a clone isolated from a primary culture of KDH-8 tumor cells by limiting dilution. The clone has been maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum and 0.584 mg/ml of L-glutamine (growth medium).

Helper-independent F-MuLV was molecularly cloned in pBR322. Molecular construction of the subgenomic viral expression vector was performed according to standard techniques.[4] The plasmid containing a *Hin*dIII-*Ban*III fragment of FV-*env* gene[5] (pZip-FV-*env*) (Fig. 1) was kindly donated by Dr. A. Ishimoto (Laboratory Gene Analysis, Department of Viral Oncology, Kyoto University). The pSV2-Neo plasmid containing the geneticin-resistance gene Neo^r was originally supplied by Dr. M. Green, St. Louis University, Missouri. Our protocol for transfection was based on the method of van der Eb *et al.*,[6] with some modifications. Briefly, subconfluent cultures of 2×10^5 cKDH-8 cl-11 cells in 60 mm petri dishes were transfected with a solution containing a DNA-calcium phosphate coprecipitate of pZip-FV-env (1 μg) or pSV2 Neo (1 μg) with salmon sperm DNA (19 μg) and left for 20 min at room temperature. The growth medium was then added to the undetached cells and incubated at 37° for 4 hr in a 5% CO_2 incubator. After removal of the medium containing the residual DNA-calcium phosphate coprecipitate, the

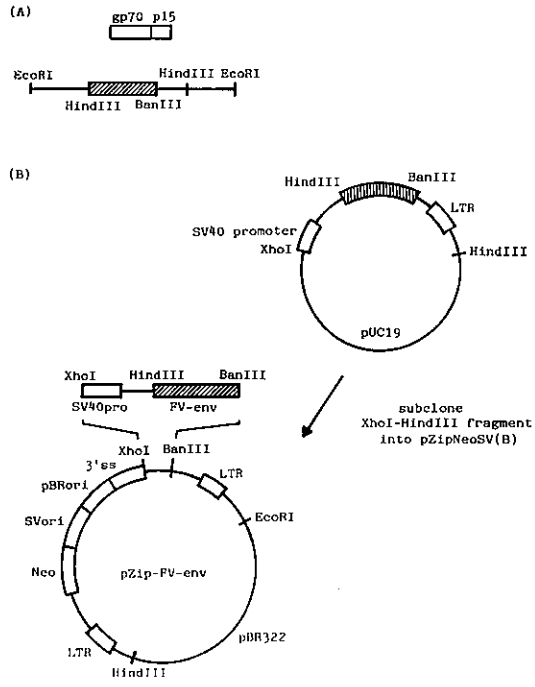

Fig. 1. A) Schematic representation of the *env* polypeptide and F-MuLV DNA. B) Molecular construction of the FV-*env* expression vector.

cells were washed twice with growth medium and were then incubated in the same growth medium for an additional 24 hr. Thereafter, the cells were subcultured in three petri dishes. After 16 hr of further incubation, the growth medium was replaced by a selective medium containing 800 μg/ml geneticin (G418 GIBCO) and further maintenance was carried out in a selective medium (400 μg/ml of G418) throughout the remainder of this study. Geneticin-resistant colonies were pooled, expanded *in vitro*, and then used as uncloned cells in this study.

Friend virus complex-infected cKDH-8 cl-11 (FV-cKDH-8) was obtained by intraperitoneal passage of cKDH-8 cl-11 tumor cells in FV-tolerant WKA/Hok rats which had been injected FV at birth and were in a viremic state.

The antiserum to the virus-associated antigen (VAA) of FV was obtained from hyperimmune-syngeneic rats by subcutaneous inoculation of 5×10^7 FV-induced tumor cells (WLFT-6) 6–8 times weekly. Diluted anti-VAA serum ($\times20$) was used for measurement of the intensity of FV envelope antigen on FV-*env* gene-transfected cells by means of indirect immunofluorescence assay (FA) with fluorescein isothiocyanate-conjugated (FITC) goat anti-rat IgG serum. The fluorescence intensities were measured by using a FACScan (Becton Dickinson).

To examine tumorigenicity of the FV-*env* gene-transfected tumor cells, various doses of the cells were inoculated into normal or immunosuppressed rats. To make rats immunosuppressed, normal rats were irradiated with γ-rays (600 rad, cobalt-60, Toshiba KXC-18-2) and were rescued by intravenous transplantation of 2×10^7 bone marrow cells from normal rats. The animals were subcutaneously injected with 1×10^6 tumor cells on the following day.

To estimate the immunogenic potential of the FV-*env* gene-transfected tumor cells, rats immunized with 1×10^6 FV-env cKDH-8 were challenged with 1×10^4 to 1×10^6 parent cKDH-8 cl-11 cells two weeks after the immunization.

As shown in Fig. 2, we confirmed the expression of FV-*env* gene product on the cell surface of FV-env cKDH-8 cells using the indirect immunofluorescence staining technique. The parent cKDH-8 cl-11 and Neo cKDH-8 did not exhibit VAA. The mode of the fluorescence intensity of FV-env cKDH-8 was, however, less than that for FV-cKDH-8 cells, the mode values being 39.81 and 79.08, respectively.

We then compared the difference in tumorigenicity between parent tumor cells and transfected tumor cells. Table I shows the tumorigenicity of cKDH-8 cl-11 and FV-env cKDH-8 cells in terms of the number of tumor cells required for a 50% lethal dose in rats (LTD_{50}). The original high tumorigenic potential ($LTD_{50}<1\times10^3$) of cKDH-8 cl-11 was greatly reduced to a value of $LTD_{50}>1\times10^6$ after FV-*env* gene transfection or FV infection, while the tumorigenicity of Neo cKDH-8 did not change in syngeneic normal rats.

To investigate whether any host-mediated immunity was involved in the regression of FV-env cKDH-8 and FV-cKDH-8 tumors, normal rats were γ-ray-irradiated (600 rad,

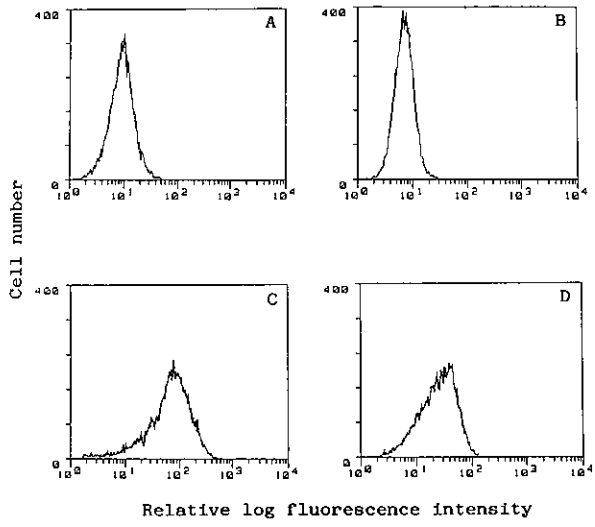

Fig. 2. Detection of VAA on the surface of FV-*env*-transfected cells and FV-infected cells by flow cytometry. A, Parent cKDH-8 cl-11 tumor cells (mode=10.09), B, Neo^r gene-transfected cKDH-8 cl-11 cells (mode =6.54); C, FV-infected cKDH-8 cl-11 cells (mode = 79.08); D, FV-*env*-transfected cKDH-8 cl-11 cells (mode=39.81). Labeled cells (1×10^4) were subjected to cytofluorometry; relative fluorescence intensity and cell number are presented in arbitrary units.

Table I. Reduced Tumorigenicity of FV-*env*-transfected cKDH-8 cl-11 Tumor Cells in Syngeneic WKA Rats

Tumor cells used	No. of rats died/No. of rats used					Tumorigenicity LTD_{50}[a] $\times10^3$
	No. of tumor cells sc challenged					
	1×10^3	5×10^3	1×10^4	1×10^5	1×10^6	
cKDH-8 cl-11	4/5	7/10	8/9	5/5	5/5	<1
Neo cKDH-8	3/5	NT[b]	4/5	5/5	5/5	<1
FV-env cKDH-8	NT	NT	NT	NT	2/15	$>10^3$
FV-cKDH-8	NT	NT	NT	NT	0/5	$>10^3$

a) The number of tumor cells required for 50% lethal growth in rats.
b) NT, Not tested.

Table II. Tumorigenicity of FV-*env*-transfected or FV-infected Tumor Cells in Immunosuppressed WKA Rats

Rats treated with	Tumor cells[a]	No. of rats died/ No. of rats used
Irradiation[b]	FV-env cKDH-8	5/5
	FV-cKDH-8	5/5
None	FV-env cKDH-8	0/5
	FV-cKDH-8	0/5

a) One$\times10^6$ tumor cells were sc inoculated into WKA rats.
b) WKA rats were irradiated with γ-rays (600 rad, ^{60}Co) one day before tumor inoculation.

cobalt-60) and were subsequently inoculated with 1×10^6 tumor cells. Table II shows that FV-*env* gene-transfected tumor cells could be rejected in normal rats but not in immunosuppressed rats.

We next examined whether the rats which were able to reject viable FV-env cKDH-8 tumor cells were also able to induce antitumor immunity against parental non-transfected tumor cells. Following the inoculation of syngeneic rats with 1×10^6 FV-*env*-transfected cells, the animals were subcutaneously challenged with parent cKDH-8 cl-11 cells. We found that the rats which had rejected FV-env cKDH-8 tumor cells were also powerfully resistant to the parent tumor cells (Table III). We therefore conclude from these observations that immunization with viable FV-env cKDH-8 cells is able to elicit strong tumor transplantation resistance to the parent tumor cells.

Table III. Incidence of Anti-tumor Immunity against Parent cKDH-8 cl-11 Tumor Cells after Immunization with Viable FV-*env*-transfected Tumor Cells in WKA Rats

Tumor cells[a] used for immunization	Lethal growth of cKDH-8 cl-11 (No. of rats died/No. of rats used)		
	No. of cells sc inoculated		
	1×10^4	1×10^5	1×10^6
None	5/5	5/5	5/5
FV-env cKDH-8	0/5	1/5	2/5

a) One $\times 10^6$ viable tumor cells were sc inoculated into WKA rats and parent cKDH-8 cl-11 tumor cells were sc inoculated after two weeks.

Many tumors show little or no antigenicity and have little inherent potential to elicit immune responses against tumor transplantation. Various experimental attempts to modify tumor cell surface structures have resulted in increased antigenicity which is able to elicit antitumor immunity.[7-9] We have already shown that a strong antitumor immunity is elicited in rat tumor cells after artificial infection with FV.[10] Because F-MuLV is of murine origin, it is advantageous to use heterologous rat tumor cells for transfection with viral *env* gene to avoid interference from endogenous viruses.

Our results show that FV-*env* gene product can be expressed on tumor cell surfaces after the gene transfection and that the FV-*env* gene product itself has the potential to reduce tumorigenicity and elicit antitumor immunity after the rejection of FV-*env* -transfected cells in normal hosts. There were no significant differences in the amounts of native class I MHC antigens (RT-1^k) of the various tumor cells in transfected or FV-infected tumor cells used in this experiment (data not shown). This might be explained by the fact that host-mediated immunity was induced by the newly expressed viral *env* gene product on tumor cell surfaces, since FV-env cKDH-8 cells were able to grow in immunologically suppressed rats but not in normal rats, while Neo cKDH-8 lost none of its tumorigenic properties in normal hosts.[11] Among 15 rats inoculated with FV-*env*-transfected tumor cells, two failed to reject the tumor, contrary to our expectations. These induced tumors were surgically removed and were checked for the presence of FV-*env* gene product on their cell surfaces. Upon examination, it was found that these tumor cells did not express detectable FV-*env* gene product on their cell surfaces (data not shown). This suggests that the decreased tumorigenicity was caused by immunological host reaction against FV-*env* gene product and that a certain quantity of FV-*env* gene product might be necessary for tumor rejection.[12] On expressing the FV-*env* gene product, xenogenized tumor cells may engender a response not only to FV-*env* gene product but also to their tumor-associated transplantation antigens through helper antigen mechanisms controlled by cytotoxic T lymphocytes.[13]

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