ISOLATION OF SARCOMA VIRUS FROM NONINBRED RATS

Z. I. Merekalova, N. P. Mazurenko*,
N. A. Khar'kovskaya, and L. D. Mezentseva

UDC 616-006.3.04-022.7:
578.828.3/-092.4-078

KEY WORDS: sarcoma virus; rats; tissue culture

Data on the presence of genetic information of type C RNA-viruses in rat cells were published in the 1970s, and later a type C retrovirus was found in cells of chemically induced rat tumors [3, 9]. More recently spontaneous replication of a retrovirus in rat cells in tissue culture has been observed [1, 4, 5]. It was not until 1978, during co-culture of embryonic Sprague-Dawley rat cells producing endogenous RLV virus with cells of various cultures transformed by chemical carcinogens, that rat sarcoma virus was isolated [7, 8]. The ability of vaccinia virus (VV) to induce malignant transformation of rat cells in vitro and replication of endogenous rat virus was discovered in the writers' laboratory. Injection of these cells into noninbred young rats led to the development of tumors at the site of injection.

The aim of this investigation was to discover whether oncogenic virus may be present in rat cells transformed by VV.

EXPERIMENTAL METHOD

Tissue culture cell line ETR (embryonic tissue of noninbred rats), undergoing malignant transformation under the influence of VV [2], and cell line ETR-M — ETR cells infected with Mazurenko leukemia virus — were used. To determine the presence of retrovirus in the tissue culture cells the method of infection centers was used. Cells of strain F-81 of cat embryonic fibroblasts, productively transformed by MO-MSV, were used as indicator cells. Cell-free materials from tumor tissue were prepared by destruction of a 50% cell suspension by triple freezing (liquid nitrogen) and thawing or by ultrasonic treatment six times, for 15 sec each time with an amplitude of 6-7, followed by centrifugation at 5,000 rpm for 1 h. Filtrates were obtained by filtration through millipore filters (0.22 μ). To study their tumorigenic properties, cell-free tumor materials and tissue cultures were injected into noninbred young rats, newly born and aged 2 weeks; the latter were irradiated beforehand with a single dose of γ-rays of 300-400 rad per rat (3-4 Gy). Blood for investigation was taken from the orbital sinus of the rats. Internal organs and tumors of the rats for histological study were fixed in 10% formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Foci of stratified cell growth were formed in the tissue culture of ETR cells after their treatment with mitomycin C and combined culture with F-81 cells in medium containing 2 μg/ml of Polybrene and 0.2 μg/ml of dexamethasone. In other words, the presence of endogenous virus was constantly detected in ETR cells by the infection centers method starting with the 23rd passage. Attempts were made to transfer the virus thus found from ETR cells into cells of a culture of normal embryonic rat fibroblasts. For this purpose, cells of a primary culture of rat fibroblasts were infected with filtrates of culture fluid of an ETR culture (85th and 101st passages), and then transferred in vitro. At the 5th-6th passage in cells of these cultures changes similar to those in cells of the ETR culture were found, i.e., the infected cells became transformed. Consequently, the retrovirus isolated from ETR cells can replicate in normal

*Deceased.

Laboratory of Viral Carcinogenesis, Laboratory Animals Department, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. A. Zedgenidze.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 476-478, October, 1988. Original article submitted December 14, 1987.

0007-4888/88/0010-1477$12.50

TABLE 1. Tumorigenic Properties of Materials Tested

Material	Passage	Rats		Incubation period of tumor development, months
		newborn	irradiated with γ-rays	
Culture fluid of ETR culture	29	$^{0}/_{10}$	$^{6}/_{8}$ (75%)	10
	44	$^{0}/_{8}$	$^{4}/_{4}$ (100%)	9
	59—60	—	$^{10}/_{12}$ (83,3%)	7
Cell-free material from rat tumors		$^{15}/_{57}$ (26,3%)	$^{12}/_{24}$ (50%)	2—4 (newborn) 1—2 (irradiated with γ-rays)
Cell-free material from culture 28		$^{0}/_{7}$	$^{11}/_{45}$ (24,4%)	2—4
Filtrate of culture fluid of culture 28		—	$^{3}/_{24}$ (12,5%)	4—5
Control		$^{0}/_{26}$	$^{1}/_{83}$ (1,2%) $^{6}/_{83}$ (7,2%)	4 10

Legend. Numerator — number of animals with tumors, denominator — total number of animals.

rat cells, can be maintained by in vitro passage, and can induce their transformation. Attempts to culture the virus in mouse and hamster cell cultures were negative.

To rescue or activate the rat retrovirus, cells of transformed ETR cultures at the 65th passage were infected with Mazurenko leukemia virus. This culture was named ETR-M. The cell culture exhibited a mixed type of growth of fibroblast-like cells with islets of epithelial cells and transplanted well in vitro. Starting with the 5th passage, ETR-M cells gave a positive XC test. Culture fluid of the ETR-M culture induced leukemia development in newborn CC57Br mice (Mazurenko leukemia) and tumor formation in young rats irradiated with γ-rays. The incubation period of tumor development in the rats was shortened during successive passage of the cells in vitro (Table 1). Cell-free material from rat tumors induced tumor development at the site of injection in γ-irradiated (50%) and newborn rats (26.3%) in the course of a short incubation period (Table 1) but did not cause the development of tumors or leukemia in CC57BR and BALB/c mice.

Rat tumor cells were cultured in vitro and transplantable cell line 28 was obtained. Injection of these cells into young rats led to tumor formation in 100% of the animals but caused no changes of tumor nature in CC57BR and BALB/c mice. Cell-free materials from a culture of line 28, like filtrates of the culture fluid of these cultures, when injected into γ-irradiated rats, induced formation of tumors similar to those which developed after injection of cell-free material from the ETR-M culture (Table 1). After subcutaneous injection of the material tumors were formed at the site of injection, and after intraperitoneal injection, tumor nodules appeared in the peritoneal cavity. At autopsy hemorrhagic ascites was found in the peritoneal cavity together with multiple tumor nodules of different sized, adherent to nearly all the organs. Distant metastases were not found, except in one case (metastases in the lung). Microscopic investigation revealed a typical fibrosarcoma, whose morphological picture varied from differentiated, with abundant collagen fibers, to almost undifferentiated. In one case a combination of a fibrosarcoma with a lymphosarcoma of the thymus and of a Peyer's patch was observed, and in two other cases a fibrosarcoma was combined with a papillary cystadenocarcinoma, metastasizing to the testes and liver.

The culture fluid from an ETR-M culture and cell-free material from rat tumors and cells of a culture of line 28, when injected into γ-irradiated young rats, thus induced the formation of analogous tumors.

It was shown (by the method of infection centers) that an endogenous rat retrovirus is present in ETR cells and can replicate in the cells of primary embryonic rat tissue. Infection of ETR cells with Mazurenko leukemia virus may perhaps have led to the formation of a virus causing fibrosarcoma in noninbred rats. The obtaining of rat sarcoma virus by recombination of endogenous rat virus with the sic genome, obtained from cells transformed by a carcinogen [7], has been described in the literature. In our own experiments a different situation occurred — rat sarcoma virus was probably formed as a result of recombination of sarcomatous virus-specific sequences obtained from rat cells after malignant transformation by VV and by a helper virus (Mazurenko leukemia virus). The isolated virus was not highly pathogenic for rats and gave weak growth when cultured in tissue culture cells. With respect

to these properties it resembled rat sarcoma virus RASV isolated from inbred animals [7, 8]. Incidentally, the virus was isolated from noninbred rats. We are aware of only one report of isolation of a nononcogenic type C virus from wild rats after treatment of the animals with methylcholanthrene [6].

The results thus demonstrate that the genome of endogenous retrovirus is potentially active in embryonic cells of noninbred rats. As in the majority of inbred rats the virus is repressed, but it can be activated by vaccinia virus and, as a result of recombination, rat sarcoma virus can be formed.

LITERATURE CITED

1. F. I. Kudryavtsev, Viruses of Cancer and Leukemia [in Russian], Moscow (1979), pp. 182-184.
2. Z. I. Merekalova and N. P. Mazurenko, Éksp. Onkol., 7, No. 6, 20 (1985).
3. V. V. Bergs, G. Pearson, H. C. Shopra, and W. Furner, Int. J. Cancer, 10, No. 1, 165 (1972).
4. K. Igarashi, R. Sasado, J. Niyama, et al., Microbiol. Immunol., 23, No. 1, 12 (1979).
5. S. Rasheed, I. Bruszewski, R. W. Rongey, et al., J. Virol., 18, No. 2, 799 (1978).
6. S. Rasheed, H. P. Charman, and M. B. Gardner, Virology, 89, No. 2, 605 (1978).
7. S. Rasheed, M. B. Gardner, and R. J. Huebner, Proc. Natl. Acad. Sci. USA, 75, No. 6, 2972 (1978).
8. S. Rasheed and H. A. Young, Virology, 18, No. 1, 219 (1982).
9. S. A. Schwartz, S. Panem, L. Stafanski, and W. H. Kirsten, Cancer Res., 34, 2255 (1974).

DETECTION OF DIFFERENTIATION FACTORS IN MALIGNANT HUMAN MELANOMAS

T. A. Zershchikova, L. N. Zaitseva,
A. V. Rodionov, M. E. Bogdanov,
and O. A. Khoperskaya

UDC 616-006.81.04-076.5

KEY WORDS: induction; differentiation factors; melanoma

Melanoma is one of the most malignant human tumors [1]. The low adhesiveness of cells of the primary tumor and invasion of thin-walled blood vessels enable it to metastasize rapidly. The primary tumor of a melanoma is small in volume and is distinguished by the high heterogeneity of its cell composition [1, 3]. Active melanogenesis takes place in the cells of this tumor, and it is probably the incompleteness of this process which determines its high degree of malignancy [1, 2]. Several workers have investigated dependence of melanogenesis on the influence of surrounding tissues [7, 13, 14], but the concrete mechanism of induction of melanogenesis has been identified by the use of the periodic albinism mutation in clawed frogs (Xenopus) genus [8, 9]. These investigations showed that melanogenesis is induced on the basis of the action of a specific melanogenic factor of protein nature, synthesized by certain meseodermal derivatives and, in particular, by the axial complex [9-11].

The aim of this investigation was to detect differentiation factors (DF) in human melanomas, and to compare them with factors acting in nonpigmented tumors, and also with certain factors acting during embryogenesis.

EXPERIMENTAL METHOD

Three sources of DF were used: human melanomas (I and II) cloned in nude mice, human papillomas, and also wild-type (+/+) Xenopus embryos at the stage of neurula and early tail

N. I. Vavilov Institute of General Genetics, Academy of Sciences of the USSR. N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 478-480, October, 1988. Original article submitted December 17, 1987.