[CANCER RESEARCH 36, 128–131, January 1976]

Comparison of Nude Mice with the Host Species for Evaluation of the Tumorigenicity of Guinea Pig and Hamster Cells Transformed *in Vitro* by Chemical Carcinogens

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SUMMARY

Nude mice (nu/nu) were compared with the species of origin for determination of the tumorigenicity of cells from chemical carcinogen-transformed and noncarcinogenic chemically treated, nontransformed guinea pig and Syrian hamster cultures. The incidence and time of appearance of progressively growing tumors were similar in the host species and in nude mice after injection of 10^7 transformed cells. Inoculation of 10^8 nontransformed cells routinely was nontumorigenic in the host species and in nude mice. The nude mouse has potential as a sensitive and reliable alternative host to the species of origin to evaluate the tumorigenicity of xenogeneic mammalian cells from cell culture model systems of carcinogenesis.

INTRODUCTION

A number of animal model systems have been developed for the study of chemical carcinogenesis in cell culture (1, 3, 4, 7–9, 21, 22). The unequivocal criterion for neoplastic transformation in these systems is the development of progressively growing tumors following inoculation of the transformed cells into recipients of the host species. Assessment of the tumorigenicity of transformed human cells will require alternative methods. Animal systems need to be developed that are suitable for the evaluation of tumorigenic potential of xenogeneic animal and human cells.

The neoplastic growth of xenogeneic tumor explants has been described in immunosuppressed subhuman primates (12), hamsters (12), and mice (13), and in congenitally immunodeficient nude mice (5). The sensitivity and reliability of these methods need to be established by simultaneous studies in recipients of the host and in xenogeneic species.

The thymusless nude mouse is potentially well suited as an alternate host to the species of origin to demonstrate the tumorigenic potential of transformed cells. The homozygous nude (nu/nu) mouse (11) is deficient in thymus-derived lymphocytes, has a decreased cellular-mediated immune response, and accepts tissue grafts from other species without the usual subsequent rejection of the foreign tissue (17, 18). Normal avian, reptilian, and human skin (16, 19) and various human fetal tissues (15), for example, survive indefinitely after transplantation to the nude mouse. Primary transplants of human adenocarcinoma of the colon, epidermoid carcinoma of the skin, and malignant melanoma (14), as well as a variety of cultured human and animal neoplastic cells (5, 6), produce progressively growing invasive tumors when transplanted to the nude mouse. Rare spontaneous tumors have been observed in nude mice housed in conventional animal facilities (10, 20); the incidence of spontaneous cancer, predominantly of lymphoreticular origin, in germ-free nude mice approximates 2% at 35 weeks (10). The low incidence of spontaneous tumors and the ability of nude mice to support the growth of a variety of transplanted normal and malignant cells make these animals a good resource for the evaluation of the tumorigenic potential of transformed cells. In this study, the tumor incidence resulting from *in vitro* chemical carcinogen-transformed and control guinea pig or Syrian hamster cells after inoculation into the host species or nude mice is compared.

Received July 21, 1975; accepted October 1, 1975.

MATERIALS AND METHODS

Cell Cultures. Guinea pig and hamster cells were derived from strain 2 guinea pig and Golden Syrian hamster fetuses. The cells were treated shortly before or after introduction into culture with a carcinogenic or noncarcinogenic chemical, observed for the appearance of morphological transformation, and tested for their ability to produce progressively growing tumors in irradiated guinea pigs or hamsters as previously described (2, 4). Vials containing 1×10^6 cells in 2 ml of medium with 10% FBS[1] and 10% dimethyl sulfoxide had been preserved in liquid nitrogen for up to 1 year before use in the present investigation.

Six guinea pig cell cultures were selected for examination. Three contained morphologically transformed cells that produced sarcomas in syngeneic guinea pigs: B(a)P culture 104C1, 19th subculture; MNNG culture 107C3, 17th subculture; and DENA culture HM2C1, 25th subculture. Three guinea pig cultures contained nontransformed, nontumorigenic cells: acetone culture 103, 40th subculture; acetone culture 118, 73rd subculture; and pyrene culture 119, 44th subculture. Six hamster cultures each at the 10th subculture were selected. Three were morphologically transformed cells and produced sarcomas in Syrian ham-

[1] The abbreviations used are: FBS, fetal bovine serum; B(a)P, benzo(a)pyrene; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DENA, diethylnitrosamine; PS, propane sultone; ENU, ethylnitrosourea; DPA, diphenylamine; NDA, *p*-nitrosodiphenylamine; Phe, phenanthrene.

sters: PS culture N4; B(a)P culture E4; ENU culture A1. Three cultures were nontransformed, nontumorigenic cells: Phe culture S2; DPA culture T2; NDA culture U3.

Vials of cells were removed from liquid nitrogen. The cells were quickly thawed at 37°, washed twice with medium containing 10% FBS, and those from each vial were transferred to a 100-mm diameter plastic Petri dish with 15 ml medium containing 10% FBS. Guinea pig cells were cultivated in Roswell Park Memorial Institute Medium 1640 with 10% FBS in a water-saturated atmosphere of 5% CO_2 in air at 38.5° (4). Hamster cells were grown in Dulbecco's modification of Eagle's minimal essential medium with 10% FBS in a water-saturated atmosphere of 10% CO_2 in air at 37° (2). Cells were maintained in continuous multiplication by serial subculture, transferred into disposable glass roller bottles within 3 to 5 subcultures, and subsequently were harvested by trypsinization and resuspended in medium without FBS for inoculation into animals (4).

Animals. Syngeneic strain 2 guinea pigs from the NIH colony and Golden Syrian hamsters from the Lakeview Colony (Charles River Lakeview, Newfield, N. J.), were housed in conventional cages on pine sawdust bedding and provided laboratory chow and tap water *ad libitum* (4). Two-day-old or 6-week-old weanlings were inoculated s.c. in the cervicodorsal region with 10^5 to 10^8 cells in 0.1 ml of medium. Weanling hamsters and guinea pigs were administered a midline total-body dose of 450 R irradiation 24 hr prior to cell inoculation (4). Irradiation was delivered at 40 rads/min, integrated midline tissue dose (tissue to air factor of 0.93 for guinea pigs and 0.98 for hamsters) from opposing ^{60}Co sources at the Armed Forces Radiobiological Research Institute, Bethesda, Md. Weanling nude mice and newborn guinea pigs and hamsters were not irradiated. Inoculated newborn guinea pigs and hamsters were weaned at 4 weeks of age. Four- to 6-week-old male homozygous nude (nu/nu) mice derived from nu/NIH Swiss parents were obtained from C. T. Hansen, Veterinary Resources, Division of Research Services, NIH. Nude mice were housed in groups of 6 animals per 11.5- x 7.25- x 5-inch standard polycarbonate animal cage in the same conventional animal facility as the guinea pigs and hamsters. Each mouse cage contained pine sawdust bedding and mouse chow. The cage was covered by a stainless steel metal screen fitted with a sheet of disposable filter paper. Each mouse cage with bedding, food, and filter top was autoclaved as a closed unit before use. A 4-oz water bottle containing tap water and fitted with a rubber stopper and metal nipple was autoclaved separately, and the metal nipple was inserted through the filter top of the cage at the time of use.

Nude mice were handled with sterile forceps and gloves; no aseptic precautions were taken when handling guinea pigs and hamsters. Guinea pig cages were changed 3 times a week. Hamster and nude mouse cages were changed twice a week. Under these conditions, the survival of guinea pigs and hamsters given injections of medium only or of nontumorigenic cells is 100% at 1 year and greater than 90% at 2 years. Survival of nude mice in our animal facility is 100% at 6 months and approximately 90% at 1 year. No grossly detectable neoplasm has been observed in 100 control animals of each of the 3 species during these periods of observation.

Animals inoculated with cells were examined twice a week for the appearance of a progressively growing tumor. Tumor-bearing animals were autopsied when moribund. Tumor-free animals were autopsied 1 year after cell inoculation.

RESULTS AND DISCUSSION

The tumorigenicity of chemical carcinogen-transformed and noncarcinogenic chemical-treated nontransformed guinea pig or hamster cells is similar in the host species and in the nude mouse. Guinea pig cells that produced progressively growing tumors in recipient guinea pigs also were tumorigenic in nude mice (Table 1). The incidence of tumors resulting from inocula of 10^5, 10^6, or 10^7 cells was identical in newborn and sublethally irradiated weanling guinea pigs. All nude mice that received 10^7 transformed guinea pig cells developed tumors. The incidence of tumors was less in nude mice than in guinea pigs following the injection of 10^5 or 10^6 cells from several transformed cultures. The time of appearance of a progressively growing tumor was similar in guinea pigs and nude mice after inoculation with 10^7 cells, approximately 1 week for B(a)P 104C1, 3 weeks for MNNG 107C3, and 5 weeks for DENA HM2C1 cells. The latent periods were the same following injection of 10^6 cells but increased 2- to 3-fold after injection of 10^5 cells. Tumors appeared at the site of cell injection and grew as large as 70 mm in diameter in guinea pigs and 50

Table 1

Comparative tumorigenic potential of cultured guinea pig cells in syngeneic guinea pigs and in nude mice

Guinea pig cells	No. of cells inoculated	Tumorigenicity[a] in Newborn guinea pig	Irradiated weanling guinea pig	Nude mouse
B(a)P 104C1	10^7	3/3	3/3	3/3
	10^6	3/3	3/3	3/3
	10^5	3/3	3/3	0/3
MNNG 107C3	10^7	3/3	3/3	3/3
	10^6	3/3	3/3	1/3
	10^5	3/3	3/3	0/3
DENA HM2C1	10^7	3/3	3/3	3/3
	10^6	3/3	3/3	0/3
	10^5	2/3	3/3	0/3
Acetone 103	10^8	0/3	0/3	0/3
	10^7	0/3	0/3	0/3
	10^6	0/3	0/3	0/3
	10^5	0/3	0/3	0/3
Acetone 118	10^8	0/3	0/3	0/3
	10^7	0/3	0/3	0/3
	10^6	0/3	0/3	0/3
	10^5	0/3	0/3	0/3
Pyrene 119	10^8	0/3	0/3	0/3
	10^7	0/3	0/3	0/3
	10^6	0/3	0/3	0/3
	10^5	0/3	0/3	0/3

[a] The number of animals developing a progressively growing tumor per number of animals inoculated.

mm in nude mice. Tumors were locally invasive with occasional grossly visible metastasis to regional lymph nodes, but no macroscopic distant metastases were observed. Inoculation of as many as 10^8 nontransformed guinea pig cells from cultures that did not produce tumors in recipient guinea pigs also did not grow as tumors in nude mice during the 1-year observation period.

The tumorigenic potential of chemical carcinogen transformed and noncarcinogenic chemical-treated nontransformed Syrian hamster cells was also similar in recipient hamsters and nude mice (Table 2). Cells from all 3 transformed cultures were tumorigenic in newborn and sublethally irradiated weanling hamsters and in nude mice following injection of 10^7 cells. Two cultures, B(a)P E4 and ENU A1, were less tumorigenic in nude mice and also produced a considerable difference in tumor incidence in newborn compared with weanling hamsters after injection of 10^5 or 10^6 cells. Cells from 1 culture, PS N4, produced tumors in each hamster and nude mouse inoculated with 10^5, 10^6, or 10^7 cells.

The time of palpable tumor appearance was similar in hamsters and nude mice and did not correlate completely with the difference in tumor incidence in hamsters and nude mice observed with 2 of the transformed cell cultures. Palpable tumors were detectable at approximately 1, 6 to 7, and 4 to 12 weeks following injection of 10^7 PS N4, B(a)P E4, and ENU A1 cells, respectively. The latent period was similar for both species after injection of 10^6 cells but increased 2- to 3-fold following inoculation with 10^5 cells. The size of developing tumors and metastatic pattern in hamsters and nude mice was similar to that described above for tumorigenic guinea pig cells.

Table 2

Comparative tumorigenic potential of cultured Syrian hamster cells in Syrian hamsters and in nude mice

Hamster cells	No. of cells inoculated	Tumorigenicity[a] in Newborn hamsters	Irradiated weanling hamsters	Nude mice
PS N4	10^7	5/5	3/3	3/3
	10^6	15/15	3/3	3/3
	10^5	12/12	3/3	3/3
B(a)P E4	10^7	5/5	3/3	1/3
	10^6	0/10	3/3	0/3
	10^5	0/3	3/3	0/3
ENU A1	10^7	4/4	3/3	2/3
	10^6	0/3	2/3	3/3
	10^5	0/3	0/3	0/3
Phe S2	10^8	0/3	0/3	1/3
	10^7	1/6	0/3	0/3
	10^6	0/4	0/3	0/3
	10^5	0/9	0/3	0/3
NDA U3	10^8	0/3	0/3	0/3
	10^7	0/6	0/3	0/3
	10^6	0/13	0/3	0/3
	10^5	0/4	0/3	0/3
DPA T2	10^8	0/3	0/3	0/3
	10^7	0/8	0/3	0/3
	10^6	0/9	0/3	0/3
	10^5	0/4	0/3	0/3

[a] The number of animals developing a progressively growing tumor per number of animals inoculated.

With 2 exceptions, inoculation of as many as 10^8 noncarcinogenic chemical-treated nontransformed hamster cells that did not produce tumors in a hamster also did not produce tumors in nude mice. The exceptions were the appearance of a tumor in 1 hamster at 6 months and in 1 nude mouse at 4 months following injection of Phe S2 cells. The tumors arose at the site of cell injection. Spontaneous tumors in Syrian hamsters and nude mice are rare during the 1st 6 months of life. Two possible explanations for the unexpected results are: (*a*) 2 roller bottle cultures of Phe S2 cells were contaminated with tumorigenic cells during cultivation or (*b*) neoplastic transformation occurred in the 2 roller bottle cultures during incubation or following injection of the cells.

The results of this investigation indicate that conventionally housed nude mice compare favorably with newborn or irradiated weanling recipients of the host species in assessing the tumorigenicity of carcinogenic or noncarcinogenic chemical-treated guinea pig or Syrian hamster cells. The incidence of progressively growing tumors and the latent period between time of cell injection and appearance of palpable tumors may vary considerably from one culture to another. For each culture, however, tumor incidence and time of appearance are similar in nude mice and recipients of the host species following injection of 10^7 transformed cells. In addition, although some cell cultures exhibit a lower incidence of tumors in nude mice and in newborn compared with irradiated weanling host recipients when 10^5 or 10^6 cells are inoculated, these differences are greatly reduced or eliminated following injection of 10^7 cells. Thus when sufficient cells are inoculated to overcome the variation in tumorigenic cells characteristic of different cultures, the results are similar in nude mice as compared with newborn or irradiated weanling recipients of the host species.

Tumor appearance usually occurs by 3 months after cell injection, an observation period associated with rare spontaneous tumor appearance in guinea pigs, hamsters, and nude mice. Inoculation of 10^8 cells that fail to produce progressively growing tumors in recipients of the species of origin are usually nontumorigenic in nude mice.

The nude mouse appears to be a sensitive and reliable alternative host to the species of origin for assessment of the neoplastic potential of xenogeneic mammalian cells in cell culture model systems of carcinogenesis.

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