Transplantation of Human Malignant Mesothelioma into Nude Mice

A. Philippe Chahinian, Jiri T. Beranek, Yasunosuke Suzuki, J. George Bekesi, Lawrence Wisniewski, Irving J. Selikoff, and James F. Holland

Department of Neoplastic Diseases [A. P. C., J. T. B., J. G. B., J. F. H.], Environmental Sciences Laboratory [Y. S., I. J. S.], and Cytogenetic Laboratory [L. W.], Mount Sinai School of Medicine of the City University of New York, New York, New York 10029

ABSTRACT

Human pleural malignant mesothelioma was successfully transplanted into nude mice from 2 of 3 patients. The tumor implants of the first generation grew in 6 of 20 mice (30%), with a take of implants of 17 of 32 (53%). Overall, tumors grew from 52 of 80 mice (65%) in a total of 169 of 266 implants (64%) during the first four generations. The mean delay between transplantation and tumor growth was 46 days (range, 18 to 104 days). Pathological examination by light and electron microscopy confirmed the nature of the growing tumors in nude mice. Pathology of transplanted tumors was grossly similar to the human tumors in both first- and second-generation transplants. Up to eight generations have been presently carried out with presence of a human karyotype in transplanted tumors. The potential usefulness of this model with particular reference to chemo sensitivity of these tumors will be investigated.

INTRODUCTION

The incidence of HMM is expected to rise in parallel with the increased use of asbestos. Few data are available regarding the efficacy of chemotherapeutic agents in HMM, and no controlled trials have been conducted yet. The lack of a suitable animal model has precluded experimental research on the treatment of mesothelioma. Induction of mesothelioma in rodents by asbestos fibers requires long periods of observation, usually more than 7 months.

During the past few years, a number of human malignant tumors have been successfully transplanted into nude mice (5–7, 9). They provide a useful tool in cancer research, since transplanted tumors retain their functional and morphological features during several generations (5, 9). Furthermore, they are easily measurable and can provide accurate information on the chemosensitivity of the human tumor (3, 5). HMM has heretofore not been successfully transplanted into nude mice.

MATERIALS AND METHODS

Tumors. In the present study, fresh HMM specimens were obtained from 3 patients with pleural malignant mesothelioma during surgical exploration. The specimens were immediately placed in ice-cold medium (RPMI Medium 1640; Grand Island Biological Co., Grand Island, N. Y.) and returned to the laboratory within 30 min. The cold specimens were trimmed of connective and adipose tissues under sterile conditions and were cut into pieces measuring 1 to 2 mm for transplantation.

Animals. Eight- to 10-week-old homozygous nude (nu/nu) male or female mice of BALB/c background were used. They were obtained from the Life Science Laboratories of the Mammalian Genetics and Animal Production Section, National Cancer Institute, Bethesda, Md., and were the outcome of sixth back-cross cycles developed by breeding the heterozygous female with nude homozygous males. The animals were housed in a laminar-air-flow room at 20–28°C under aseptic conditions. Filtered air and sterilized bedding, cages, food, and water were used.

Transplantation. Under light anesthesia by inhalation with methoxyflurane (Penthrane; Abbott Laboratories, North Chicago, Ill.), tumor specimens were surgically transplanted either s.c. (1 to 2 pieces/site) into 1 to 4 sites/mouse in the groin and axilla, or i.p. under aseptic conditions. A trocar was used in some experiments. The mice were observed every weekday, and the tumors were measured as soon as growth occurred. The largest diameter (length) and shortest diameter (width) were measured weekly with vernier calipers and corrected for thickness of the skin. Tumor volume was calculated for a prolate ellipsoid, using the formula

\[ V = \frac{\pi}{6} LW^2 \]

Mice were weighed weekly.

Examination of Transplanted Tumors. Tumors were surgically excised, using light anesthesia with methoxyflurane, and were prepared for light and electron microscopy according to techniques previously described (12). Light microscopy sections were stained with hematoxylin-eosin, periodic acid-Schiff with and without diastase digestion, and Mowry's colloidal iron with and without hyaluronidase digestion for hyaluronic acid detection (12). Electron micrographs were taken by a Siemens type 101 electron microscope.

Tumor cell suspensions were prepared from xenografts in nude mice for karyotyping. After surgical excision of the tumor, fatty and hemorrhagic tissues were dissected away, and the specimen was finely minced with sharp scissors in ice-cold RPMI Medium 1640 at pH 7.3. The resulting material was incubated at 37°C with 20 ml of RPMI medium containing 10% FCS (Grand Island Biological Co.) and 10 mg collagenase (Millipore Corp., Freehold, N. J.; 155 units/mg) in a trypsinizing jar with constant stirring. At the end of 45 min of incubation.
the suspension was filtered with 2 layers of sterile gauze and then centrifuged at 1000 × g for 10 min at room temperature. The cell pellet was then resuspended in RPMI medium containing 10% FCS and centrifuged again. This was repeated 3 times. The final pellet was resuspended in RPMI medium with 10% FCS. Final concentration of viable cells was 3 to 4.5 × 10⁶ cells/5 ml. The 5-ml suspension was exposed to 0.075 mg of vinblastine sulfate (Eli Lilly and Co., Indianapolis, Ind.) in 0.15 ml of 0.9% NaCl solution at 37° for 2 hr. The cells were then treated with a hypotonic solution (0.053 M KCl:0.013 M sodium citrate, 1:1) for 12 min and fixed in 3 changes of methanol:acetic acid (3:1). Air-dried slides were prepared and stained with quinacrine HCl (Sigma Chemical Co., St. Louis, Mo.).

RESULTS

For the first generation, a total of 20 mice were used (Table 1). Transplantation was successfully achieved from 2 of 3 patients. None of the i.p. transplants grew. For s.c. implants, the period between transplantation and detectable tumor growth had an average of 46 days (range, 18 to 104 days). Some mice died prematurely for various reasons (wasting syndrome, infections). No specific cause of death was found at autopsy in these mice, and in particular there was no pathological evidence of murine hepatitis infection. Overall results up to 4 generations are presented in Table 1. Chart 1 shows the tumor growth of 4 s.c. implants which followed a Gompertzian-like pattern. There was no obvious difference in take rates between male and female nude mice, although the majority of mice were females (Table 1).

Histocytological studies of the transplanted tumors of the first generation from Patient 2 (a 44-year-old woman without asbestos exposure) revealed large round neoplastic cells and a poorly developed network of connective tissue containing capillaries. Glycogen was detected in the cytoplasm, and hyaluronic acid was occasionally identified in either cell cytoplasm or extracellular space. These characteristics were similar to those of the original pleural tumor, which was evaluated as a malignant mesothelioma, epithelial form (Fig. 1A). The transplanted tumors of the second generation from Patient 2 were histologically identical (Fig. 1B). Electron microscopy confirmed the epithelial nature of these cells, with well-developed microvilli on the cell surface, intracellular vacuoles with short microvilli, aggregated bundles of tonofilaments, and an incomplete basement membrane (Fig. 2, A and B). In the transplanted tumors from Patient 3 (a 56-year-old man with asbestos exposure), the majority of tumor cells were found to be of epithelial type, although a small number of spindle-shaped cells were also found. Glycogen was detected in the cell cytoplasm. Hyaluronic acid was also detected mainly in the extracellular space. Histological findings were confirmed by electron microscopy and were similar in both the original human tumor and the first- and second-generation xenografts.

Up to 8 generations have been presently carried out with evidence of a human karyotype in transplanted tumors (Fig. 3).
Human Mesothelioma in Nude Mice

Fig. 1. Light microscopy. A, original human mesothelioma (Patient 2). A sheet-like arrangement of epithelial cells is seen. One mitosis is present (arrow). H & E, x 430. B, transplanted tumor in nude mouse (second generation, Patient 2). Large round neoplastic cells showing a sheet-like arrangement. Infiltration of mononuclear cells is seen in the periphery of the tumor. H & E, x 430.

DISCUSSION

Many different human tumors have been transplanted into nude mice (6, 7, 9). To our knowledge, successful transplantation of human malignant mesothelioma has not been previously achieved. Wynn-Williams and McCulloch (14) attempted such a transplantation into nude mice and observed that "growth occurred to a slight extent." The success rate was high (65% of mice and 64% of implants) and tumors grew readily as shown in Chart 1. Light and electron microscopic studies revealed close similarities with the original human biopsy.

HMM is still a rare tumor in clinical practice. The availability of this model can allow the study of chemotherapeutic sensitivity of these tumors, with obvious practical consequences for clinical trials. Furthermore, the mean delay of appearance and growth of tumors of 46 days is much shorter than that for mesotheliomas caused by injection of asbestos fibers into rodents. This should allow the determination of chemosensitivity of a tumor in real time, with potential usefulness for the treatment of the corresponding patient. Further studies are in progress to determine the effect of various chemotherapeutic agents on transplanted human mesothelioma.

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REFERENCES

Fig. 2. Electron microscopy. A. original human mesothelioma (Patient 2). Epithelial cell with large number of mitochondria (M), intracytoplasmic vacuoles (IV), and microvilli (M). × 16,000. B. transplanted tumor in nude mouse (second generation, Patient 2). Well-developed microvilli (M), large number of mitochondria, and an incomplete basement membrane (BM) are shown. × 11,500.
Fig. 3. Q-banding karyotype of transplanted tumor in nude mouse. (Patient 2, seventh generation). Ten metaphases of suitable quality were analyzed, and they contained 43 chromosomes, most of which were clearly human in morphology and banding. In all 10 cells, 13 autosomes and 1 sex chromosome were replaced by 11 marker chromosomes (M) varying in size and centromeric index. Marker chromosomes replaced both members of Pairs 17, 19, and 22; 1 member of pairs 1, 3, 4, 7, 9, 13, and 14; and 1 sex chromosome.
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