Transplantation of Human Tumors in Nude Mice

Yukio Shimosato, Toru Kameya, Kanji Nagai, Setsuo Hirohashi, Tsutomu Koide, Hiroatsu Hayashi, and Tatsuju Nomura

SUMMARY—Ninety-one human tumors, including various common carcinomas, low-grade malignant tumors, and benign tumors, were transplanted into athymic nude mice. Tumor take was confirmed histologically for 22 neoplasms at the initial transplantation, and 14 serially transplantable tumors were established, including some hitherto unestablished or unreported, such as lung and hepatic cell carcinomas. Among the 91 tumors were 21, 14, and 13 carcinomas of the lung, stomach, and breast, respectively. Transplantability was highest in lung carcinomas (10/21), followed by gastric carcinomas (2/14) and breast carcinomas (1/13). Morphology of original tumors was retained well in most transplanted tumors, but desmoplastic or scirrhous tumors, such as gastric and breast carcinomas, tended to become medullary with a decrease in amount of tumor stroma. The ability to produce mucin in gastric carcinomas or melanin in malignant melanoma was maintained in serially transplantable tumors. In addition, ectopic production of adrenocorticotropic and beta melanocyte-stimulating hormone continued in a transplanted small cell carcinoma of the lung. Preliminary results were obtained on hormone dependency of the transplantable breast carcinoma and on \(\alpha\)-fetoprotein in the transplantable hepatic cell carcinoma.


Since the report of Rygaard and Povlsen (1), human cancers have been transplanted into athymic nude mice by many investigators. Their studies have shown the usefulness of the animals and transplantable tumors and have brought out several problems (2–17). We began transplanting human tumors into nude mice in December 1973 and established 14 serially transplantable human cancers, including hitherto unestablished or unreported tumors. Here we report our results of transplantation. We describe morphologic and other characteristics of transplanted tumors and various problems related to tumor transplantation in nude mice; details of specific transplantable tumors, such as hormone-producing or -dependent tumors, will be reported separately.

MATERIALS AND METHODS

Tumors.—As shown in table 1, 91 human tumors of various kinds, obtained at surgery or autopsy between December 1973 and June 1975, were used for transplantation.

Animals.—Animals used were 4- to 7-week-old, male or female nude mice with the genetic background of BALB/c, which were bred and maintained in flexible vinyl film isolators under the specific pathogen-free (SPF) conditions at the Central Institute for Experimental Animals, Kawasaki, Japan. The source, breeding, rearing, and microflora of SPF nude mice were described previously (18).

For most experiments, mice were kept in cages placed in a flexible vinyl film isolator, but in a few instances they were in cages covered by paper-filters placed in a laboratory free from other animals. In both cases, mice were fed freely with sterilized water and CLEA #2 chow for germfree mice (Nihon CLEA Co., Tokyo, Japan).

Examination of transplanted tumors.—At the time of passage, tumors and animals were examined grossly; tissues, by a light microscope. An electron microscope was used when needed. For light microscopy, tissues were fixed in formalin and sections were stained with hematoxylin and eosin or alcian blue-periodic acid-Schiff. For electron microscopy, minced tissue fragments were doubly fixed with glutaraldehyde and osmium tetroxide; they were embedded in Epon 812, and ultrathin sections were stained with uranium and lead.

According to the type of tumor, tumor tissue and serum were assayed for specific substances. Bioassay and radioimmunoassay for antidiuretic hormone (ADH) were performed by Dr. T. Kimura (Department of Internal Medicine, Tohoku University Hospital) and by Dr. N. Ohsawa (Department of Internal Medicine, Tokyo University Hospital), respectively. Radioimmunoassays for ACTH and beta melanocyte-stimulating hormone (\(\beta\)-MSH) were done by Dr. K. Abe (Endocrine Division, National Cancer Center). Radioimmunoassays for Au antigen and \(\alpha\)-fetoprotein were done by Dr. T.
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Skin Malignant melanoma

Soft tissue Rhabdomyosarcoma

Salivary gland Carcinomas

Stomach Adenocarcinoma, solid

Tongue Squamous cell carcinoma

Testis Germ cell tumor

Eye Retinoblastoma

Liver Liver cell carcinoma

Prostate Adenocarcinoma

Hypophysis Adenoma

Mediastinum Thymic tumor

Kidney Clear cell carcinoma

Nasal sinus Carcinoid tumor

Colon Adenocarcinoma

Thyroid Papillary, clear cell, follicular, and medullary carcinomas.

Embryonal carcinomas and malignant teratoma.

Adenoid cystic and acinic cell carcinomas.

Thymoma and thymic carcinomas.

successful serial transfers are listed in table 1 according to

Transplantability of Tumors

The number of tumor takes and the number of successful serial transfers are listed in table 1 according to the primary sites and histologic types of tumors.

Lung carcinomas

Twenty-one lung carcinomas consisted of 4 squamous cell carcinomas, 7 adenocarcinomas, 6 small cell anaplastic carcinomas, 3 carcinoid tumors and 1 adenoid cystic carcinoma. Among these, growth was confirmed in 10 tumors (10/21, 47.6%). Of the 74 mice used, 21 (28.4%) showed tumor growth. Serial passage was successful in 5 of 7 attempted; 1 moderately differentiated squamous cell carcinoma (Lu-9), 1 poorly differentiated adenocarcinoma (Lu-14), and 3 small cell carcinomas [2 intermediate type (Lu-1 and 19) and 1 oat cell type (Lu-24)], which were in the second to seventh passage. Total serial passage rate of common lung carcinomas, excluding carcinoid tumor and adenoid cystic carcinoma, was 5/17 (29.4%).

Although tumor growth was noted in an adenoid cystic carcinoma of low-grade malignancy at the initial transplantation, none of 3 well-differentiated adenocarcinomas of broncholoalveolar type grew in mice.

Gastric carcinomas

Of 14 tumors used for transplantation, 9 were solid and obtained either from serosal aspects of the primary tumor or from the lymph node metastasis (3 well-differentiated and 3 moderately differentiated papillary or tubular adenocarcinomas and 3 poorly differentiated signet-ring cell type scirrhous carcinomas). Two of the 9 solid tumors grew in mice (in 3 mice among 27 mice receiving transplants). One was a poorly differentiated signet-ring cell type scirrhous carcinoma (St-4), which was in the ninth passage at the time of this report; it grew rapidly and reached an average of 500 mm² in a month with 100% take rate. Another was a moderately differentiated tubular adenocarcinoma (St-15) obtained from a patient with α-fetoprotein in serum; it was in the first passage and grew slowly, reaching 300 mm³ in 2 months.

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TABLE 1.—Transplantability of human tumors

<table>
<thead>
<tr>
<th>Primary site</th>
<th>Histology</th>
<th>Number of tumors transplanted</th>
<th>Number of tumor takes</th>
<th>Number of serially transplantable tumors/No. attempted</th>
<th>Number of transfers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>Squamous cell carcinoma</td>
<td>4</td>
<td>2</td>
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<td>5</td>
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<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>7</td>
<td>3</td>
<td>1/2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Small cell carcinoma</td>
<td>6</td>
<td>4</td>
<td>3/3</td>
<td>7,2,2</td>
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<td>Carcinoid tumor</td>
<td>3</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
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<tr>
<td></td>
<td>Adenoid cystic carcinoma</td>
<td>1</td>
<td>1</td>
<td>0/1</td>
<td>0/0</td>
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<tr>
<td>Stomach</td>
<td>Adenocarcinoma, solid</td>
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<td>2</td>
<td>2/2</td>
<td>9,1</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma, ascitic fluid</td>
<td>5</td>
<td>1*</td>
<td>0/1*</td>
<td>0/0</td>
</tr>
<tr>
<td></td>
<td>Leiomyosarcoma</td>
<td>1</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Breast</td>
<td>Duct carcinoma, solid fluid</td>
<td>9</td>
<td>0</td>
<td>0/0</td>
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<tr>
<td></td>
<td>Duct carcinoma, pleural fluid</td>
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<td>1</td>
<td>1/1</td>
<td>5</td>
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<tr>
<td>Liver</td>
<td>Liver cell carcinoma</td>
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<td>1</td>
<td>1/1</td>
<td>7</td>
</tr>
<tr>
<td>Colon</td>
<td>Adenocarcinoma</td>
<td>3</td>
<td>2</td>
<td>1/2</td>
<td>6</td>
</tr>
<tr>
<td>Appendix</td>
<td>Malignant carcinoid tumor</td>
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<td>0</td>
<td>0/0</td>
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<tr>
<td>Thyroid</td>
<td>Careinoma*</td>
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<td>0</td>
<td>0/0</td>
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<td>2</td>
<td>2/2</td>
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<td>Prostate</td>
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<td>Testis</td>
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<td>Ovary</td>
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<td>0/0</td>
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<tr>
<td>Uterus</td>
<td>Choriocarcinoma</td>
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<td>0</td>
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<td>Clear cell carcinoma</td>
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<td>0/0</td>
<td>0/0</td>
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<tr>
<td>Salivary gland</td>
<td>Carcinoma*</td>
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<td>1</td>
<td>0/1</td>
<td>0/1</td>
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<td>Nasal sinus</td>
<td>Malignant carcinoid tumor</td>
<td>1</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Eye</td>
<td>Retinoblastoma</td>
<td>1</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Tongue</td>
<td>Squamous cell carcinoma</td>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>4</td>
</tr>
<tr>
<td>Unknown</td>
<td>Acinic cell carcinoma</td>
<td>1</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Soft tissue</td>
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<td>0</td>
<td>0/0</td>
<td>0/0</td>
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<tr>
<td>Mediastinum</td>
<td>Thymic tumor*</td>
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<td>0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Hypophysis</td>
<td>Adenoma</td>
<td>5</td>
<td>0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
</tbody>
</table>

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* Not verified histologically.
+ Papillary, clear cell, follicular, and medullary carcinomas.
+ Embryonal carcinomas and malignant teratoma.
+ Adenoid cystic and acinic cell carcinomas.
+ Thymoma and thymic carcinomas.

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No tumor growth was observed after inoculation of well-differentiated adenocarcinoma.

In 5 cases, tumor cells were obtained from the ascites of patients and inoculated in the subcutaneous tissue of 12 mice. In 1 case, a small tumor developed in 2 of 9 mice. This was transferred to other mice without histologic verification, but the mice died from accident shortly after transfer. Tumor cells from 3 cases were inoculated into the peritoneal cavity of 4 mice, none of which showed tumor growth.

As a whole, the number of mice with histologically verified tumor take at the initial transplantation was 3 of 45 (7.0%), and serially transplantable tumors were established from only 2 of 14 gastric carcinomas (14.3%).

Breast carcinomas

All of 13 tumors were common ductal-type carcinomas. Eleven tumors were transplanted into female mice and 3 into male mice. Four of 9 solid tumors transplanted were resolved completely. Five were judged as non-take: Although nodules about 5 mm in diameter developed about 5 weeks after inoculation, they regressed gradually, completely in 2 and to minute scar-like tissue in 3 that remained 7 months after inoculation. Remains of the tumor in the scarlike tissue were confirmed histologically in one mouse, but none of those grew after passage to other mice.

In four instances, the tumor cells obtained from the pleural fluid of patients were inoculated into the subcutaneous tissue of mice. One of these (Br-10) was successfully transplanted. This tumor was derived from a patient who had developed breast cancer before menopause and undergone bilateral oophorectomy. The tumor cells were inoculated in mice 19.5 hours after thoracocentesis. The tumor was in the fifth passage with a tumor take rate of 100%; it reached about 125 mm³ in a month. The tumor cells obtained from the pleural fluid of one patient were inoculated in the peritoneal cavity of mice, but no tumor growth was observed.

As a whole, the initial tumor take was observed in 1 of 13 tumors (7.7%). Only 1 of 44 mice used showed tumor growth (2.3%).

Other tumors

One of 5 liver cell carcinomas transplanted in mice showed progressive growth (Li-4); it reached 15 mm in diameter in 14 days and was in the seventh passage, being the most rapidly growing tumor among the serially transplantable tumors in our possession.

One of 3 colon carcinomas (Co-3) and 2 of 4 malignant melanomas (Me-1 and -2) were in the sixth, sixth, and third passage, respectively. A malignant melanoma (Me-1) was a very slow growing tumor, reaching 10 mm in diameter in 5 months at the initial transplantation, but the growth rate gradually increased to 10 mm in diameter in 2 months at the third passage. Besides these, 1 squamous cell carcinoma of the tongue (To-1) and 1 poorly differentiated adenocarcinoma of the prostate gland (Pr-1) could be serially transferred.

No serially transplantable tumors were established from low-grade malignant or benign tumors. However, remains of tumor tissue in mice were histologically confirmed in one each of animals given pituitary adenoma and thymoma, 9 and 15 weeks after inoculation, respectively.

Metastasis From Transplanted Tumors

In this series, metastasis to a regional lymph node was observed only in Li-4 of liver cell carcinoma origin. This was noted 28 days after the first transfer when the transplanted tumor was 40×30×15 mm. No hematogenous metastasis was found.

Morphology of Transplanted Tumors

Serially transplantable squamous cell carcinoma of the lung (Lu-9) retained a keratotic tendency (figs. 1, 2), and the transplanted adenoid cystic carcinoma of the bronchus (Lu-3) revealed not only its characteristic histologic architecture but also the presence of well-differentiated neoplastic myoepithelial cells at the periphery of tumor nests. Small cell carcinoma of the lung (Lu-1, -19, and -24) continued to possess granules similar to neurosecretory granules in cytoplasm (figs. 3, 4).

In a gastric carcinoma (St-4), slight desmoplastic reaction in the initial transplant was noted (figs. 5, 6), but it changed to a medullary tumor with a decrease in amount of stroma occurring with the passage of the tumor. In the fifth passage, tumor cells at the periphery were larger, polygonal, and undifferentiated, with a large and prominent nucleolus (fig. 7). However, in the central portion, particularly near foci of necrosis, tumor cells frequently took a signet-ring form, with abundant intracytoplasmic mucin eccentrically displacing the nucleus (fig. 8), and occasionally formed incomplete tubules retaining the original characteristics. In another gastric carcinoma (St-15), the transplanted tumor consisted entirely of typical signet-ring type cells, although the original tumor had areas of papillary and tubular patterns in addition to signet-ring cells.

The transplanted breast carcinoma (Br-10) in the first transplant was reminiscent of metastatic foci in lymph nodes removed at the time of radical mastectomy (fig. 10); it had central scarring, peripheral trabeculae, and small nests bordered by occasional crescent cells with eosinophilic cytoplasm suggestive of a myoepithelial nature (figs. 11, 12). A few cells contained intracytoplasmic mucus, and occasional glandular structures were noted. Mucus-producing cells were also observed in smears prepared from the patient's pleural fluid used for transplantation (fig. 9). Along with passages of tumors, a tendency to central scar formation was lost, but features observed at the periphery of the initial transplant were well retained.

The serially transplantable liver cell carcinoma (Li-4) and its metastasis in a lymph node displayed features of poorly differentiated adenocarcinoma with mucus production (figs. 14, 15), but liver cell origin could be suspected in areas from the trabecular arrangement of tumor cells. At autopsy of the patient, the tumor showed typical features of trabecular type liver cell carcinoma, with areas of bile production and of glandular structures, which indicated differentiation toward bile ducts also (precisely a combined liver cell and bile duct carcinoma) (fig. 13).

Tumors derived from well-differentiated adenocarcinoma of the colon (Co-3) (fig. 16), well-differentiated squamous cell carcinoma of the tongue (To-1) (fig. 17), and poorly differentiated adenocarcinoma of the prostate gland (Pr-1) retained original morphology. One malignant melanoma (Me-1) became amelanotic after transfer, but another (Me-2) continued to be melanotic (fig. 18).
Biochemical, Endocrinologic, and Cytotoxic Characteristics of Transplanted Tumors

The patient with Lu-1, small cell carcinoma of the lung origin, presented a typical clinical syndrome with inappropriate secretion of ADH (Schwartz-Barter syndrome) but no signs of ectopic ACTH or β-MSH production. Biologic assay proved the presence of ADH (22.0 μU/mg wet wt) in the primary tumor, which was not tested for ACTH or β-MSH. The metastatic tumor developed in the subcutaneous tissue 10 months after pneumonectomy, which was used for nude mouse transplantation. It did not contain detectable ADH, but 17 ng of ACTH and 33 ng of β-MSH per gram of wet tissue were detected by radioimmunoassay. The tumor transplanted in mice at the fourth passage contained 10 ng of ACTH and 7.1 ng of β-MSH per gram of wet tissue, but no ADH was detectable by radioimmunoassay. Inasmuch as ACTH and β-MSH were undetectable in the normal liver of the mouse, rat, and human, and from the assay methods employed here (22, 23), these values were considered to be significant, indicating good preservation of ability to produce peptide hormones ectopically in the transplanted tumor in nude mice. Despite the presence of immunoreactive ACTH in the transplanted tumor, adrenal glands of mice bearing the tumor were not hypertrophied.

For the breast carcinoma (Br-10), the growth rates of the tumor were compared in female mice and in male mice with or without im injections of 0.1 mg estradiol dipropionate in oil, which were given once a week for 5 weeks starting 5 weeks after transplantation of the tumor. Ten weeks after transplantation, the tumor size in 6 female mice was 106±41 mm³ (mean ± sn), whereas that in 2 untreated male mice was 25 and 35 mm³ and that in 2 male mice treated with estradiol was 200 and 84 mm³, respectively. This suggested that Br-10 tumor growth was hormone dependent; it was retarded in male compared with female mice and accelerated in male mice treated with estradiol. The transplanted tumor was also positive for estrogen binding protein.

In transplantable amelanotic melanoma (Me-I), tyrosinase activity was detected in Golgi apparatus and abortive premelanosomes by electron microscopic cytochemistry (fig. 19), although typical premelanosomes were not found.

Serum of the patient with hepatic cell carcinoma Li-4 contained Au antigen and 420 μg/ml of αf-fetoprotein, but the tumor did not produce detectable Au antigen or αf-fetoprotein in sera of the tumor-bearing mice, as assessed by radioimmunoassay. The transplanted tumor, however, showed positive immunofluorescence in a few cells, i.e., several cell clusters in a 10×15-mm section of the tumor, with rabbit antiserum to αf-fetoprotein (fig. 20). Control sections that reacted with normal rabbit serum or rabbit antiserum to ACTH as the first antibody were negative for immunofluorescence. αf-Fetoprotein could not be detected in sera of mice bearing gastric carcinoma St-15. The sections of this tumor also failed to react with rabbit antiserum to αf-fetoprotein by the indirect immunofluorescence method.

DISCUSSION

Ninety-one tumors have been transplanted into nude mice, the tumor take was confirmed in 22, and 14 serially transplantable tumors were established. The tumor take rate was lower than expected and lower than that of cancer cells maintained in vitro (10, 17). The low tumor take rate could be explained by the following: 1) tumors used for transplantation included many of low-grade malignancy and many that were benign; 2) inoculum size was sometimes small for both solid and fluid tumors, especially in the beginning of the experiment; 3) many mice (≥30% in the first year but much fewer in the second year) died of “wasting disease,” or by accident due to leaks of water from the drinking bottle.

Transplantability of carcinomas of the lung, stomach, and breast was highest for lung and lowest for breast carcinomas. None of 3 well-differentiated adenocarcinomas of bronchioalveolar type grew in mice, although in two instances, mice fed under conventional conditions died of unknown cause within 34 and 47 days after transplantation. Therefore, the transplantability of this tumor must be reexamined.

The transplantability of gastric carcinoma might not be so low, since Ueyama and Tamaoki (personal communication) established 4 serially transplantable gastric carcinomas including well-differentiated papillary and tubular adenocarcinomas of the intestinal type. The low transplantability of desmoplastic or scirrhous carcinomas, such as gastric and breast carcinomas, might be due to the presence of human connective tissue preventing contact of tumor cells with tissues and body fluids of mice. However, of 5 gastric carcinomas in ascites form there was only one take, which was lost at the second passage. Of 4 breast carcinomas from pleural exudate, one take was obtained in a single mouse.

All attempts to obtain transplantable tumors in nude mice in an ascitic form from 1 breast and 3 gastric carcinomas have failed so far. However, we recently succeeded in establishing a transplantable breast carcinoma in ascites form from the cell line (Hattori) established and maintained in vitro for several months by Minato et al. (24). It was in the third passage and killed all the mice in a month or so. The ascites form of the tumor is between a solid tumor system and an in vitro cell line; it is obviously a useful tool for certain experiments.

In 5 breast carcinoma cases, the tumors decreased in size gradually after they had reached 5 mm in diameter. Mechanisms of possible spontaneous regression have to be studied along with the animals’ hormonal status influencing tumor growth.

Tissues from one case each of thymoma and pituitary adenoma remained in the subcutis of nude mice without growth for 15 and 9 weeks, respectively. This phenomenon is assumed to be analogous to a take of normal human tissues such as skin (25) and gonads (26).

Growth of initially transplanted tumors was generally slow, except for liver cell carcinoma Li-4. Along with serial transfers of the tumor, the tumor take rate increased almost to 100% in many instances, and growth became faster, as in the case of Me-1. Increase in rates of transplantability and growth is assumed to be due largely to adaptation of tumor cells to the mouse environment, since morphologic characteristics of tumors are well preserved.

Metastasis was found only for Li-4 of the present series, but it was detected occasionally in mice that had received transplants of tumor cells maintained in vitro (10, 16, 17). Although metastases were said to be uncommon in cases of direct transplantation of human tumors (2, 3, 11, 17), Giovanella and co-workers (10) reported a

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case of pharyngeal carcinoma, and Ueyama and Tamaoki (personal communication) experienced one example of gastric adenocarcinoma and uterine cervical squamous cell carcinoma among 19 transplantable tumors. Therefore, one should observe the mice long enough, particularly for tumors of slow growth.

Morphologic characteristics of original tumors were usually maintained well, as reported by previous investigators (2, 3, 6, 10, 11, 14, 15, 17). A decrease in the amount of tumor stroma observed in St-4 and Br-10 may be due to lack of reaction of mouse fibroblasts to human tumor cells, to growth speed of the tumor, or to absence of lymphatic invasion by the tumor in mice. Study along this line may elucidate mechanisms of demoplastia in some human cancers. Anaplastic tendency observed in Li-4 may be due to rapid growth or to lack of alignment of mouse endothelial cells with tumor cells.

It has been reported that functions of tumors were also maintained in nude mice, i.e., production of human chorionic gonadotropin and placental alkaline phosphatase by the SCH line (16, 17). It is noteworthy that ectopic production of ACTH and \( \beta \)-MSH was also maintained in Lu-1, that tyrosinase activity could be demonstrated in Me-1 even after it became amelanotic, and that growth rate of Br-10 was high in female mice and in male mice treated with estradiol. However, in Li-4, no \( \alpha \)-fetoprotein could be detected in sera of tumor-bearing mice, although the tumor used for initial transplantation should have been checked for those antigens. The positive fluorescence for \( \alpha \)-fetoprotein in sections will be re-evaluated along with the assay of the tumor extract. The carcinoembryonic antigen (CEA) was described to be maintained in transplanted colon adenocarcinoma (11, 13). Recently, Miwa (personal communication) detected CEA in colon adenocarcinoma Co-3, the values of which roughly paralleled the tumor size. The tumor possessing marker substances such as hormones and enzymes can be used not only for evaluating results of chemotherapy, radiotherapy, and immunotherapy, but also for the study of relationship between tumor growth and differentiation.

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FIGURES 1, 2.—Histology of Lu-9, moderately differentiated squamous cell carcinoma of lung. Tumor used for transplantation (fig. 1) and 3d-passage tumor in mice with focus of keratinization at left upper corner (fig. 2). × 175

FIGURES 3, 4.—Ultrastructures of Lu-1, small cell carcinoma of lung. Tumor used for transplantation (fig. 3) and 1st-passage tumor in mice (fig. 4). Both contain neurosecretory type granules in cytoplasm. × 17,000
Figures 5-8.—Histology of St.4, signet-ring cell gastric carcinoma. Desmoplastic tumor used for transplantation (fig. 5); initial transplant in mice with signet-ring cells and slight desmoplasia (fig. 6); periphery of 5th transfer tumor in mice with sheets of undifferentiated cells without desmoplasia (fig. 7); and center of 5th transfer tumor with numerous signet-ring cells (fig. 8). × 175
FIGURES 9-12.—Cytology and histology of Br-10, duct carcinoma of breast. Tumor cells in pleural fluid used for transplantation with a few mucin-containing cells (fig. 9). × 600. Lymph node metastasis removed from patient by radical mastectomy (fig. 10). × 175. Initial transplant with central scarring (fig. 11). × 175. Initial transplant with peripheral trabecular arrangement (fig. 12) identical to that seen in figure 10. ×175
Figures 13-15.—Histology of Li-4, liver cell carcinoma. Tumor at autopsy of patient: Liver cell carcinoma in trabeculae (Fig. 13A) and tubule formation (Fig. 13B); 2d-transfer tumor in mice with features of poorly differentiated adenocarcinoma (Fig. 14); lymph node metastasis at 2d passage in mice; solid growth fills peripheral sinusoid (Fig. 15). × 175

Figure 16.—Histology of Co-3, well-differentiated adenocarcinoma of colon at 2d transfer in mice. × 175
Figure 17.—Histology of To-1, squamous cell carcinoma of tongue with keratotic pearl at 2d passage in mice. × 175

Figure 18.—Histology of Me-2, malignant melanoma at 1st passage in mice, with melanotic tumor cells and melanophores. × 350

Figure 19.—Ultrastructural cytochemistry of Me-1, amelanotic melanoma at 4th passage, with tyrosinase activity in Golgi vesicles and abortive premelanosomes. × 42,000

Figure 20.—Immunohistochemistry for αfetoprotein of Li-4 at 2d passage, with positive fluorescence in tumor cell cytoplasm. × 350