HETEROTRANSPLANTATION OF HUMAN NEOPLASMS IN NUDE MICE

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ABSTRACT

Heterotransplantation of human neoplastic tissue was investigated in the congenitally athymic mice, nude mice, BALB/c-nu/nu, kept in SPF condition within vinyl isolator.

At present, 23 out of 32 established cell lines of various malignant neoplasm, originated from the lung, uterine cervix, endometrium, breast, cholangioma, neuroblastoma, osteosarcoma and giant cell tumor have been successfully grafted in nude mice.

Thirty six out of 162 surgically removed materials including carcinoma of the stomach, lung, kidney, maxilla, endometrium, uterine cervix, ovary, choriocarcinoma, meningioma and neuroblastoma have been transplanted in nude mice.

The neoplasms serially transplanted in nude mice showed similar histological characteristics of the primary tumors.

Endocrine activities were also well preserved in nude mice, as revealed by the elevated blood HCG levels and histological changes in uterus and ovary of the host nude mice grafted with choriocarcinoma, and by erythropoiesis in the spleen of mice grafted with renal cell carcinoma which is producing erythropoietin. The grafted neoplasms showed similar susceptibility to anti-cancer agents as originated one.

INTRODUCTION

Heterotransplantation of human neoplasms to experimental animals is usually followed by rejection of the tumor grafts unless the host has been rendered immunologically unresponsive with thymectomy, irradiation, antilymphocytes serum treatment etc.

Even in these cases, serial transplantation is difficult.¹ The congenitally

athymic mouse, nude mouse, deprives the thymus dependent immune system, especially transplantation immunity.^{2,3} Successful heterotransplantation of human tumors and cultured human tumor cells to the nude mice has been previously reported by Rygaard and Povlsen,^{4,5} and Giovanella *et al.*⁶ The histology of the tumor heterografts proved to be identical to that of the original tumors^{4,5,7} and transplanted tumors preserved their chromosome types⁸ and carcinoembryonic antigen producing activity in rectum carcinoma.⁷

Since October 1973, we have tried heterotransplantation of 162 specimens of human neoplasms from both tissue culture lines and surgically operated cases to nude mice. This paper presents the results of the heterotransplantation of human neoplasms and the characteristics of transplanted tumors.

MATERIALS AND METHODS

Nude mice: BALB/c-nu/nu maintained under SPF condition within vinyl isolator.

Surgical specimens: Tumor specimens were obtained by surgery and biopsy, and tissue fragments were inoculated subcutaneously with trocar into the nude mouse within 1 to 12 hours following surgical excision.

Cell lines: 32 established cell lines maintained by Dr. Ohboshi, National Cancer Center were used. The tumor cells obtained by trypsinization of cell monolayers were injected subcutaneously into the back of nude mice (about $1-5 \times 10^7$ cells per mouse).

Human Chorionic Gonadotropin (HCG) assay: Heparinized blood was obtained from retro-orbital venous plexus once a week. HCG contents were assayed by a specific radioimmunoassay for HCG.

Bleomycin and Mitomycin: Bleomycin was purchased from Nihon-kayaku Co., Ltd. Mitomycin from Sankyo Co., Ltd.

Schedule for therapy assay of Bleomycin and Mitomycin using the lung cancer grafted in nude mice (Fig. 1):

All test animals were inoculated with about $3 \times 3 \times 3$ mm³ of solid tumor tissue from the same donor. The tumor transplant was placed subcutaneously on the right side of the flank. The animals were weighed twice a week and tumor dimensions were measured in two planes with a slide gauge. When the tumor size exceeds 30 mm² (about 30 days after inoculation) treatment was started. Total dose 50 mg Bleomycin and total dose 5 mg Mitomycin was administered intraperitoneally 2 times/2 days/week $\times$ 3 weeks. Animals were sacrificed 23 days later. Tumor tissues were subjected to histological examination.

1. Tumors Lung cancer (from metastasized lymph-node)
2. Animals BALB/c-nu/nu (in SPF condition)
3. Drugs Bleomycin, Mitomycin
4. Schedule for treatment

Fig. 1 Schedule for therapy assay of bleomycin and mitomycin using the lung cancer transplanted to nude mice.

RESULTS

Transplantation:

Table 1 summarizes the results of heterotransplantation of human malignant

Table 1

Transplantation of human malignant tumors to nude mice

List of successfully transplanted tumors:

1. From tissue culture	23/32
2. From biopsied or operated tumor	36/162
From human specimen	From tissue culture
Lung cancer	(5) Lung cancer
Stomach cancer	(5) Oat cell type (2)
Uterine cervix cancer	(5) Adenocarcinoma type (4)
Renal cell carcinoma	(5) Squamous cell type (1)
Melanoma	(2) Melanoma (2)
Ovarian cancer	(2) Giant cell tumor of bone (2)
Cancer of the upper jaw	(2) Uterine cervix cancer (1)
Krukenberg's tumor	(1) Endometrial cancer (1)
Endometrial cancer	(1) Endometrium (Malig, transform) (1)
Meningioma	(1) Cholangioma (1)
Osteogenic sarcoma	(1) Breast cancer (1)
Neuroblastoma	(1) Stomach choriocarcinoma (1)
Liposarcoma	(1) Neuroblastoma (1)
Testis tumor	(1) Osteogenic sarcoma (1)
Chorioepithelioma	(1) Hodgkin's disease (1)
Rhabdomyosarcoma	(1) Acute lymphocytic leukemia (1)
	Burkitt's lymphoma (1)

tumors to nude mice. Successful transplantation has been attained in 23 out of 32 cultured tumor cells and 36 out of 162 biopsied and operated tumors. Transplanted tumors in nude mice include 12 lung cancers, 6 uterine cervix cancers, 5 stomach cancers, 5 renal cell carcinomas, 2 endometrial cancers, 2 neuroblastomas, 2 choriocarcinomas and so on. The interval between the day of transplantation and the appearance of a detectable nodule was three to twelve weeks. In some cases such as neuroblastoma and acute lymphocytic leukemia (MOLT), the size of the transplanted tumor became as big as the mouse itself. Serial transplantation has been usually successful. For instance, 11 out of 12 lung cancers, 5 out of 6 stomach cancers and 5 out of 5 renal cell carcinomas have been serially maintained in nude mice. Metastases were rare in nude mice. Only three cases, 2 uterine cervix cancers and one choriocarcinoma, were observed in 59 successfully transplanted human cancers.

The histological examination revealed the close similarity of the morphology and the degree of differentiation between original and transplanted tumors. In

Fig. 2 Tumor size and HCG level in the host nude mouse transplanted with choriocarcinoma. Tumor size is indicated by tumor area (length $\times$ breadth). Broken line indicates tumor size and solid line indicate HCG level.

few cases, tumors became a little more undifferentiated during the serial transplantations.

Functional activities:

Two cases of functioning tumors, HCG producing choriocarcinoma and a erythropoietin producing renal cell carcinoma, have been chosen to analyze whether the functioning activities of human malignant tumors are maintained after the heterotransplantation in nude mice.

Fig. 2 shows the time sequences of plasma HCG and tumor size of choriocarcinoma transplanted to nude mice. As shown in the figure, until four to five weeks after the transplantation, the tumor remained unchanged in size and plasma HCG levels were undetectable. Then, the tumor started to grow with a concomitant increase in plasma HCG. Histological analysis of the nude mouse showed the exaggerated host responses to HCG. Remarkably enlarged ovaries with hemorrhage in the follicles and marked proliferation and increased secreting activity of the endometrium of the uterus have been observed.

In the case of erythropoietin producing renal cell carcinoma, the host nude mouse revealed the response to erythropoietin with reddish coloured skin due to the polycythemia.

Hematocrit and red blood cell counts in nude mice increased to 67% and 1.4×10^7 respectively. The histology of the spleen of the nude mouse showed the

Fig. 3 Therapy assay of mitomycin and bleomycin. Tumor size is indicated by tumor area (length $\times$ breadth) and is shown on the ordinate, and days of treatment are shown on the abscissa.

marked proliferation in the erythroid series.

Sensitivity to the anti-cancer drugs:

Fig. 3 shows the results of a therapy assay of Bleomycin and Mitomycin on a human lung cancer transplanted in nude mice. The test procedure is based on the schedule originally used by Povlsen and Rygaard.⁹ Tumor size is shown on the ordinate and days of treatment are shown on the abscissa. With the doses used in this experiment, Bleomycin is suppressing the growth of the tumor, whereas Mitomycin is less effective. In the clinical course, the patient with this lung cancer was refractory to the treatment with Mitomycin. The effect of Bleomycin was not fully evaluated.

DISCUSSION

Although the usefulness of heterotransplantation of human malignant tumors to nude mice has been advocated by Rygaard and Povlsen in 1967,⁵ not many results have been reported in the literature.^{4,6,7} The large scale trials of our studies on the subject presented in this paper have clearly demonstrated and confirmed the previous results that human malignant tumors are transplantable to nude mice in a considerable number of cases.

The comprehensive analysis of unsuccessful transplantation is needed to improve the procedure.

The unique features of the transplanted human malignant tumors in nude mice are the preservation of various characteristics of original tumors. In almost all cases tested, histology showed a remarkable resemblance between original and transplanted tumors. It seems to be surprising that the histology of the transplanted tumors in nude mice from tissue culture, such as a choriocarcinoma, is almost the same as that of the original tumor, even after several years of maintenance in tissue culture. In a few cases, however, histological appearance showed a slight change, especially a tendency of poor differentiation. The functional activities of functioning tumors are another example of the preservation of tumor characteristics. Our results clearly indicated that HCG producing choriocarcinoma and erythropoietin producing renal cell carcinoma are keeping their functions after serial transplantations. Transplanted tumors also seem to preserve the sensitivity to anti-cancer agents of the original tumors. In the case of lung cancer, the results obtained in the therapy assay on nude mice are similar with the clinical experiences.

These findings strongly suggest that heterografts in nude mice may be suitable models for studies of tumor immunity and chemotherapy in cancer patients and provide a reliable source of human tumor material for in vitro and

in vivo experiments.

The details of these results will be published elsewhere.

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