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Citation	泌尿器科紀要 (1980), 26(2): 119-125
Issue Date	1980-02
URL	http://hdl.handle.net/2433/122599
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

Acta Urol. Jap. Vol. 26
No. 2, February 1980

HETEROTRANSPLANTATION OF TRANSPLANTABLE HUMAN UROGENITAL MALIGNANT NEOPLASMS TO THYMECTOMIZED ANTITHYMOCYTE SERUM TREATED NEONATAL HAMSTERS

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ABSTRACT

Experiments representing an attempt to transplant human urogenital malignant tumors transplantable in nude mice to antithymocyte serum (ATS)-treated, thymectomized neonatal hamsters were conducted, with the results assessed in comparison with those in nude mice, using specimens from human anaplastic prostatic adenocarcinoma, transitional cell carcinoma of the bladder and renal cell carcinoma. These tumors transplanted grew somewhat slower in hamsters and showed a slightly pronounced tendency of adherence to surrounding tissues, compared to those in nude mice. There was no evidence of metastasis of the tumors transplanted in hamsters and nude mice.

The results of the experiments suggest possibilities of development of laboratory animals in which human malignant tumors of the urogenital system may be transplanted under ordinary conditions of feeding.

INTRODUCTION

The use of the vinyl isolator is essential for raising and carrying out experiments on nude mice but considerable cumbersomeness is inherent in it. If it be practicable to attain transplantation, especially serial transplantations, of human urogenital malignant tumors to hamsters, an extensive application of the animal to cancer research will be possible as with the nude mice.

The present study was undertaken to investigate histopathologic features of the growth in thymectomized neonatal golden hamsters injected subcutaneously with ATS of transplanted human urogenital malignant neoplasms which had been serially grafted in nude mice. The data compared with those on these transplantable tumors in nude mice have suggested potential experimental usefulness of ATS-treated, thymectomized hamsters in various fields of biological research.

MATERIALS AND METHODS

Experiments were carried out by a slight modification of the method of Levey et al.[1], using 30 adult non-inbred Syrian golden hamsters of both sexes weighing 15 to 20 g which were thymectomized at two weeks of age. Thymectomy is necessary in hamsters since the thymus is located within the thoracic cavity, where the following precautions should be observed: (1) avoid hemorrhage, (2) completely remove the thymus and (3) complete the operation as quickly as practicable.

Preparation of ATS

Ten adult golden hamsters were thymectomized and the thymus was removed aseptically, care being taken to minimize hemorrhage. The thymus tissue was placed in a sterile Petri dish containing culture medium (Ham F-12) and adjoining connective tissues, blood vessels and capsules were carefully removed. It was then minced as finely as possible and, after vigorous pipetting, passed twice filtered twice through a 80– to 150–u stainless steel mesh, and washed with three changes of culture medium by centrifugation at 1,200 rpm for 10 minutes each. The

resulting lymphocyte suspension was adjusted to a concentration of 2×10^7 cells per ml and injected into two normal albino rabbits intravenously in a dose of 10^8 cells per animal via the auricular vein. The animals received booster injections with the same numbers of hamster thymic lymphocytes for three consecutive days beginning 14 days after the initial injection. On day 7 after the final injection blood was drawn by cardiac puncture, from which serum was immediately separated, decomplemented by heating at 56°C for 30 minutes, and, after absorption with hamster erythrocytes and myelocytes, stored frozen.

Titration of ATS

Assay for cytotoxic potency of ATS was performed by the method of Sakakibara et al.[5]

To aliquots of serial twofold dilutions of antiserum (ATS), equal volumes of 1:3 dilution of lyophilized guinea pig complement in Tyrode's solution were added, followed by addition of equal volumes of target cell (hamster thymic lymphocyte) suspension previously adjusted to 5×10^5 cells per ml. The mixtures were incubated at 37°C for 45 minutes.

The mixtures were then cooled for 10 minutes to stop the reaction and spun at 1,000 rpm for 10 minutes, and the supernatant was discarded, and the cells resuspended in Tyrode's solution. The viable cell count was made of the cell suspension with 0.2% trypan blue, whereby the percentage of killed cells in the whole population calculated to determine the cytocidal potency of the ATS being defined here as reciprocal of the dilution of ATS causing death of 50% of the cell population. ATS preparations showing cytocidal potencies of $\geqq 512$ units were used in the experiments. Two-week old, thymectomized hamsters were injected with 0.5ml of ATS by subcutaneous route daily for a period of 2 weeks. Human anaplastic prostatic adenocarcinoma, transitional cell carcinoma of the bladder or renal cell carcinoma transplantable to nude mice was minced into fragments, $3 \times 3 \times 3$ mm, and transplanted subcutaneously into the back of hamsters. The animals then received ATS in amounts of 0.5 ml s.c. on alternate days for a period of 4 weeks. At 4 weeks after the transplantation the hamsters were sacrificed and examined at autopsy for growth and metastatic spread of the tumor. Histopathologic examinations were made of slides prepared from paraffin blocks, with hematoxylin and eosin or Masson-trichrome stain, of the tumor specimens fixed in 20% formalin.

RESULTS

The growth of transplanted human tumors in hamsters was measured at consecutive periods following transplantation, with the results as follows. The tumor growth rate was determined in 30 nude mice as controls, viz. 10 with anaplastic prostatic adenocarcinoma, 10 with transitional cell carcinoma of the bladder and 10 with renal cell carcinoma. The average tumor growth rate for these 30 nude mice, expressed as ratio of tumor volume at 4 weeks after transplantation to that at transplantation, was about 12 times. The average ratio was determined to be 9 times approximately for 30 hamsters. The growth rate of transplanted human tumors in hamsters varied noticeably, ranging from remarkably rapid growth (Fig. 1) to slow growth (Fig. 2).

There was gross evidence of vascularization into the tumor from surrounding tissues in the hamsters as in the nude mice.

Microscopic examinations of these transplanted tumors were carried out to compare their histopathologic features with those of tumors transplanted in nude mice.

Microscopically, anaplastic prostatic adenocarcinoma transplanted in nude mice comprised large clear cells, small, somewhat dark cells and stroma, presenting histologic features of remarkably anaplastic adenocarcinoma with only sparse tubular glandular structures, and with occasional necrotic foci possibly due to active proliferation (Fig. 3).

After transplanted in hamsters, the tumor showed little or no change in the relative subpopulations of large clear cells and small

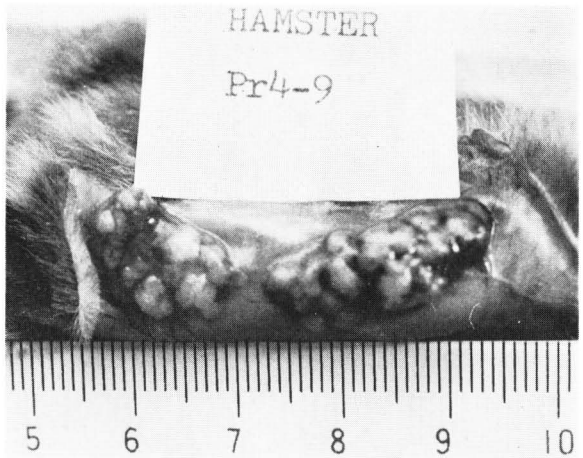

Fig. 1. Actively grown, anaplastc prostatic adenocarcinoma in hamster 4 weeks after subcutaneous transplantation of two 3×3×3 mm tumor fragments. The lobular proliferation of adenocarcinoma is well demonstrated.

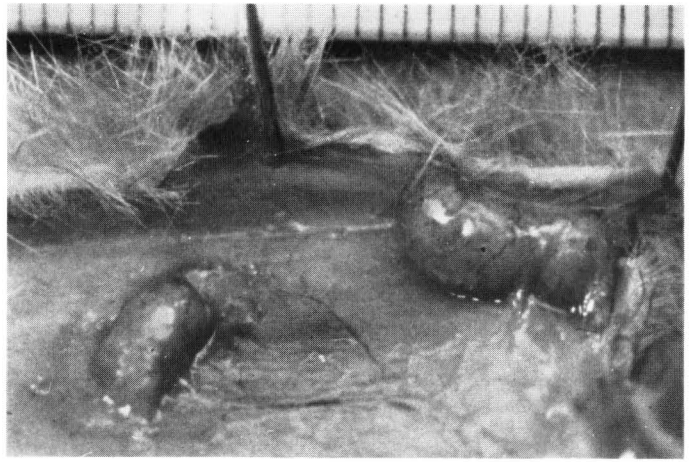

Fig. 2. Relatively slowly growing transitional cell vesical carcinoma in hamster 4 weeks after transplantation of three 3×3×3 mm fragments of tumor. Note the vascularization supplying from the surrounding tissue to the tumor.

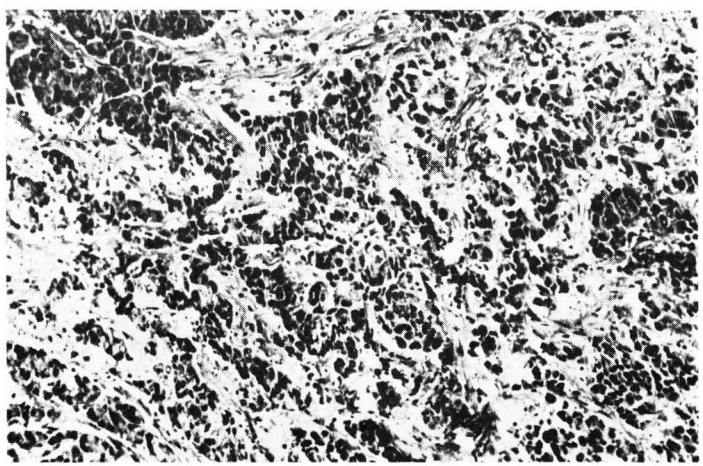

Fig. 3. Anaplastic adenocarcinoma in nude mice; remarkable anaplastic type with only sparse tubular glaudular structures and with occasional necrotic foci.

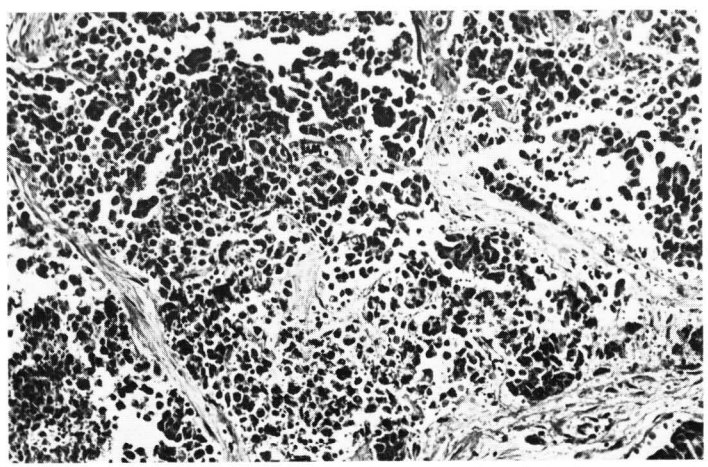

Fig. 4. Anaplastic adenocarcinoma after transplanted in hamsters; the tumor showed slight proliferation of stroma as compared to the tumor transplanted in nude mice.

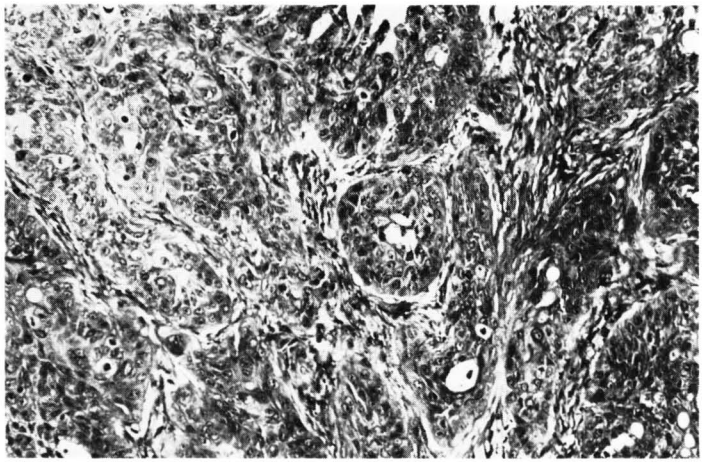

Fig. 5. Transitional cell vesical vesical carcinoma transplanted in nude mice; undifferentiated transitional cell carcinoma with adenomatous patterns.

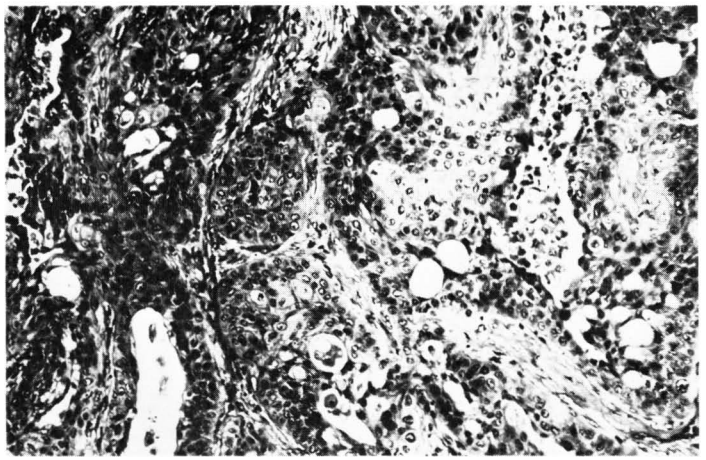

Fig. 6. Transitional cell vesical carcinoma transplanted in hamsters; the tumor showed essentially the same structures with minimal cystoid changes.

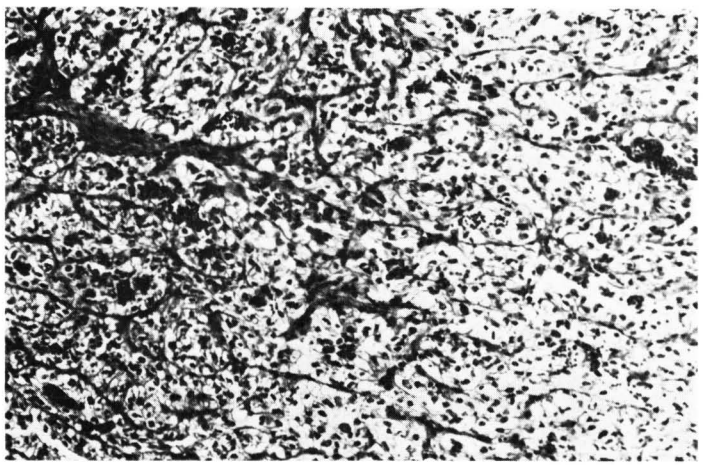

Fig. 7. Renal cell carcinoma in nude mice; transplanted a mixed type tumor consisting largely of clear cells with intermixed dark cells and scanty of stroma.

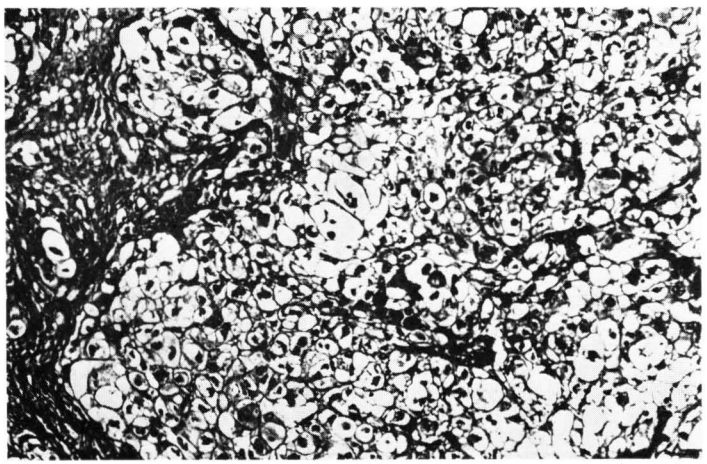

Fig. 8. Renal cell carcinoma transplanted in hamsters; virtually comparable in character to that in nude mice although it contained increased stromal proliferation.

dark cells with slight proliferation of stroma as compared to the tumor transplanted in nude mice (Fig. 4).

Transitional cell carcinoma of the bladder transplanted in nude mice had microscopic features which basically may be referred to as undifferentiated transitional cell carcinoma though containing elements that showed adenomatous patterns (Fig. 5). When transplanted in hamsters, the tumor showed essentially the same structures with minimal cystoid changes (Fig. 6).

In nude mice, transplanted renal cell carcinoma was histologically a mixed type tumor consisting largely of clear cells with intermixed dark cells and scanty of stroma (Fig. 7). The tumor transplanted in hamsters was virtually comparable in character to that in nude mice although it contained increased stromal proliferation (Fig. 8).

DISCUSSION

The optimal conditions for growth of heterotransplanted human or other animal tumors, according to Stanbridge et al.[2], are (a) neonatal thymectomy coupled with ATS or (b) thymectomy followed by whole-body irradiation and bone marrow reconstitution. They also state that the use of immuno-suppressed animals has great potential in cancer research, especially in studies with human malignant material.

Wagner and co-workers[3] prepared heterologous antilymphocyte serum (HAS) in rabbits and a pony and carried out its titration *in vivo* in terms of its ability to suppress the rejection of allografted tumors.

Meanwhile, tumor-specific transplantation antigens (TSTA) were discovered by Haughton et al.[4] in spontaneous tumors of mice, and immunization against these TSTA may render normal animals resistant to subsequent grafting with tumors from syngeneic donors.

It was also reported by Sakakibara and his associates[5] that the tumor size reached a maximum on day 21, followed by regression, in hydrocortisone-treated golden hamsters with transplanted xenogeneic tumors whereas the tumor size tended to progressively increase consistently even beyond the third week in ATS-treated animals; hence a significant effect of the administration of ATS.

In a study of Benjamin et al.[6] with materials derived from naturally occurring human bladder carcinoma, it was noted that, microscopically, the transitional cell carcinoma heterotransplanted in hamsters changed into one with spindle cell features.

Cobb[7] described the growth and metastatic spread of human tumors in immunosuppressed hamsters. In hamsters immunosuppressed by combined thymectomy and ATS treatment to which a variety of human tumors were transplanted in the flank or the cheek pouch, metastases to the lungs were evident in the period up to 4 months after grafting, with carcinomata of the breast, colon, larynx and kidney and also with melanoma, rhabdomyosarcoma, fibrosarcoma and teratoma of the testes. Pulmonary metastases were observed with 14 of 20 different tumors implanted. Only 2 tumors, a hypernephroma and a melanoma, according to the report, became established at the site of implantation whilst the remainder showed regression even if the tumor was proliferating in the lungs.

In hamsters at 2 weeks of age, thymectomy has frequently resulted in a high mortality because of the anatomical localization of the organ within the thoracic cavity. Davis et al.[8] described that thymectomy at 28 days of age significantly enhanced the immunosuppressive effect of ALS and delayed recovery from that suppression.

Experimental studies on antitumor therapy in nude mice with transplanted human tumors of the urogenital organs have been conducted at this laboratory, of which the significance has been reported[9,10].

Lewis et al.[11] attempted treatment with methotrexate against human choriocarcinoma transplanted in ALS-treated hamsters, with the finding that there was no significant difference in oncolytic effect of the drug on the tumor between ALS-treated animals and untreated animals.

Animal species subjected to heterotrans-

plantation of human tumors have been largely confined to rodents, including mice as well as hamsters.

It was reported by Arnstein et al.[12] that NIH Swiss mice treated with ATS to diminish their cell-mediated immune responsiveness proved to be gratifying as substrates for the propagation of a variety of human malignant tumors.

These observations suggest possible usefulness of other animals for heterotransplantation studies with human malignant neoplasms, among the various species of laboratory animals.

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(Accepted for publication, Sept. 13, 1979)

和文抄録

ヌードマウス可移植性ヒト泌尿生殖器腫瘍の胸腺摘出，ATS 処理ハムスターへの移植

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ヌードマウス可移植性ヒト泌尿生殖器腫瘍のうち，われわれが保持している未分化型前立腺癌，膀胱移行上皮癌，腎細胞癌について胸腺摘出後，ATS を投与した新生児ハムスターにそれぞれ皮下移植をこころみた．

これらの腫瘍は，ヌードマウスにおける継代移植時におけるときと，（1）やや発育が遅く，（2）周囲組織との癒着性，が存在しているのが相異点であった．ただし移植時に比して，あきらかに腫瘍の増大が認められ，通常飼育下で実験動物としての使用される可能性があるものと考えられた．