HETEROTRANSPLANTATION OF HUMAN GASTRIC CANCER IN NUDE MICE

BY

Shiro Izumo$^*$

ABSTRACT

Human gastric cancer was transplanted into the peritoneum of nude mice, and the progress of invasion and growth of cancer were investigated. Serial transplantations succeeded in 4 strains of human gastric cancer and one strain of canine gastric cancer induced by N-thy1-N-nitro-N-nitrosoguanidine (ENNG). The five strains grew subcutaneously in nude mice, and both single strain and mixed strain were transplanted into the mouse peritoneal cavity by a surgical procedure.

In the single strain, cancer cells demonstrated mucosal and/or submucosal invasion in the gastrointestinal tract. In the mucosal layer, cancer permeation into the lymphatic duct was verified.

In the histological examination, each strain of the mixed ones grew back to back with no interference, showing front formation. The human strain and canine strain co-existed in the mouse. A human strain of poorly differentiated adenocarcinoma showed hematogenous metastasis to the liver.

This is the first report that the invasion as one of biological characteristics of the primary human gastric cancer was clearly demonstrated, and also, another important biological characteristics i.e., the hematogenous liver metastasis was manifested in the mixed strain.

INTRODUCTION

An athymic and hairless nude (nu/nu) mouse is deficient in all thymus-derived cell-mediated immunological responses. Rygaard and Povlsen (1969) for the first time reported the transplantation of human neoplasms into nude mice which are deficient in T-cell functions. As the transplanted neoplasms in nude mice have hardly any difference from those of primary tumors, both morphologically and biochemically, much has been expected since on the transplantation in the studies on cancer. Possibly because of the still short history since its introduction into Japan, reports published to date on the uses of transplantation have been primarily related to the studies with emphasis laid upon addition of morphological studies on the subcutaneous transplantation, whereas results of experiments through other routes have not yet been fully analyzed. The subcutaneous transplantation would be more advantageous as it enables continued direct observation of the progress, but the transplanted neoplasms tend to grow in a localized manner while keeping its morphological form almost intact. The first successful grafting of neoplasms into nude mice was performed with the materials of colonic adenocarcinoma and mammary cancer, followed by the materials collected from gastric cancer. As it is relatively difficult to es-

$^*$ 出雲一郎: Ist Department of Surgery (Chief: Prof. T. Murakami, M.D.), Tokyo Medical and Dental University School of Medicine (Tokyo Ika Shika Daigaku).

Received for publication, March 16, 1979.
establish the transplanted and incubated gastric cancer strains, attention has been directed to the subcutaneously transplanted strains over multiple generations in nude mice. No patterns of infiltration or metastasis have so far been reported of the strains of gastric cancer. This would mean that a judgement that the method would result in simple establishment of strains cultured over multiple generations can be justified without any counter-argument and would lead to the possibility that an important factor in xenografting is deficient in it for use in the model experiments relative to human gastric cancer.

In order to expedite the above-mentioned aspects, we intraperitoneally transplanted the multiply transplantable strain into subcutaneous tissues of nude mice so that a more practical assessment could be made on the propagation and growth of human gastric cancer on the basis of the results of transplantation. The results revealed that metastasis and invasion of grafted gastric cancer could be clarified and that the transplanted neoplasms could be propagated in nude mice in a similar manner as in the clinical cases of human patients. A similar experiment on transplantation was carried out by mixing four strains of transplantable human gastric cancer and one strain of canine experimental gastric cancer. This experiment was intended for investigation on whether or not a large variety of transplantable strains could co-exist with no mutual interventions in a single mouse. The results of our experiment will be described hereinafter, along with some discussions.

**Materials and Methods**

1. **Nude mice**

Nude mice of both sexes of mixbreed, about 4 weeks of age, were kept in the environments close to the conventional conditions within vinyl isolators to each of which a large Cambridge microfilter was attached. The nude mice employed were the SPF ones (BALB/cA/Bom nu/nu) raised in Bomboltgard in Denmark with hereditary backgrounds of BALB/c.

2. **Materials**

Tissues of neoplasms were aseptically collected from the serosa of the operatively resected specimens of gastric cancer at our clinic. The materials for grafting were firstly rinsed in the tissue culture fluid (MEN or TC-199 containing 100 units of penicillin and 1 mg of streptomycin per milliliter) and minced as finely as possible in the tissue culture fluid. Fragments with a volume of 8 mm³ (with an edge of 2 mm) and with viable appearance were employed for a mouse. Finely minced specimens of human gastric cancer, with a volume of approx. 8 mm³, were transplanted into the flank subcutaneous space of the mice through a trocar needle. Five kinds of transplantable strains were obtained; 4 were human gastric cancer and one was canine gastric cancer induced by N-ethyl-N-nitro-N-nitrosoguanidine (ENNG). The canine experimental gastric cancer strain was prepared by applying the solution of 150 μg/ml of ENNG, 20 mg/ml of Tween 60, and 20 g of skim milk in 250 ml of water, twice a day for 12 successive months to develop gastric cancer of Borrman III Type of a size of 5×6 cm in the gastric antrum. The strains thus made transplantable were further subjected to transplantation in the subcutaneous tissues of mice.

3. **Intraperitoneal transplantation**

The transplantable strains from the subcutaneous tissues of nude mice were intraperitoneally transplanted by aseptically operated method under anesthesia with ether. The groups employed for the ex-
periment comprised the exclusive strain groups for which either of the two initially obtained strains was used, and the groups of mixed strain groups for which 4 strains of human gastric cancer with or without the canine experimental gastric cancer strain were involved.

4. Observation of grafted strains

Tumor-bearing mice were sacrificed every 4 weeks after intraperitoneal transplantation. The rate of propagation of xenografted area in the subcutaneous tissues was measured twice a week in terms of the size of (major axis × minor axis)³/₂ for knowing the volume doubling time. At autopsy, all the mice employed for the experiment were histologically examined on the photomicroscopic level. The inspected organs were lung, stomach, liver, spleen, large intestine, small intestine, mesenterium, and intrapelvic organs in addition to the transplanted tumor. Hematoxylin-Eosin staining was principally used, along with PAS, Alcian Blue, Azan-Mallory, and Masson-Trichrome as required.

RESULTS

I. Investigation on subcutaneously transplanted strains

The results of transplantation of 12 cases with human gastric cancer and 1 case with canine experimental gastric cancer into the subcutaneous space of nude mice are indicated in Table 1. The materials collected from human gastric cancer subjected to our present experiment were grossly classified into 1 case of Borrmann I type, 1 case of Borrmann II type, 9 cases of Borrmann III

Table 1.

<table>
<thead>
<tr>
<th>Number</th>
<th>Patient</th>
<th>Pathologic Number</th>
<th>Clinical Stage</th>
<th>Borrmann Type</th>
<th>Histological Type &amp; Invasive Layer</th>
<th>Tumor Infiltration</th>
<th>Site</th>
<th>Transplanted Tumor Mucosal Level</th>
<th>Transplanted Number</th>
<th>Take Size (mm²)</th>
<th>Take Time (days)</th>
<th>Number of Transfers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S.M.</td>
<td>0-62598</td>
<td>III</td>
<td>III</td>
<td>pap sse</td>
<td>β</td>
<td>V₃</td>
<td></td>
<td></td>
<td></td>
<td>76</td>
<td>3/6</td>
</tr>
<tr>
<td>2</td>
<td>W.M.</td>
<td>0-68043</td>
<td>III</td>
<td>III</td>
<td>tub-lse</td>
<td>β</td>
<td>V₁</td>
<td></td>
<td></td>
<td>93</td>
<td>783</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>I.T.</td>
<td>0-68045</td>
<td>II</td>
<td>III</td>
<td>por sse</td>
<td>γ</td>
<td>V₁</td>
<td></td>
<td></td>
<td>48</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>H.G.</td>
<td>0-68044</td>
<td>II</td>
<td>III</td>
<td>tub-lsse</td>
<td>γ</td>
<td>V₁</td>
<td></td>
<td></td>
<td>48</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>5</td>
<td>O.T.</td>
<td>0-68544</td>
<td>III</td>
<td>III</td>
<td>por sse</td>
<td>γ</td>
<td>V₁</td>
<td></td>
<td></td>
<td>78</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>6</td>
<td>O.G.</td>
<td>0-69052</td>
<td>III</td>
<td>IV</td>
<td>sig sse</td>
<td>γ</td>
<td>V₁</td>
<td></td>
<td></td>
<td>46</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>7</td>
<td>S.E.</td>
<td>0-69247</td>
<td>II</td>
<td>III</td>
<td>tub-lsse</td>
<td>β</td>
<td>V₁</td>
<td></td>
<td></td>
<td>81</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>8</td>
<td>S.S.</td>
<td>0-69636</td>
<td>III</td>
<td>III</td>
<td>por sse</td>
<td>γ</td>
<td>V₁</td>
<td></td>
<td></td>
<td>48</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>9</td>
<td>M.S.</td>
<td>0-69637</td>
<td>II</td>
<td>I</td>
<td>sig pm</td>
<td>α</td>
<td>V₀</td>
<td></td>
<td></td>
<td>41</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>10</td>
<td>K.M.</td>
<td>0-69738</td>
<td>III</td>
<td>III</td>
<td>por sse</td>
<td>γ</td>
<td>V₀</td>
<td></td>
<td></td>
<td>46</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>11</td>
<td>S.T.</td>
<td>0-72565</td>
<td>III</td>
<td>III</td>
<td>tub-lse</td>
<td>β</td>
<td>V₁</td>
<td></td>
<td></td>
<td>56</td>
<td>1840</td>
<td>(+)</td>
</tr>
<tr>
<td>12</td>
<td>F.T.</td>
<td>0-73275</td>
<td>II</td>
<td>III</td>
<td>por sse</td>
<td>α</td>
<td>V₁</td>
<td></td>
<td></td>
<td>44</td>
<td>3200</td>
<td>(+)</td>
</tr>
<tr>
<td>13</td>
<td>ENNG/4</td>
<td>No. 18</td>
<td>III</td>
<td>III</td>
<td>tub-lse</td>
<td>γ</td>
<td>V₀</td>
<td></td>
<td></td>
<td>36</td>
<td>231</td>
<td>(+)</td>
</tr>
</tbody>
</table>
Fig. 1. Volume doubling time of each strain.

type, and 1 case of Borrmann IV type. When histologically classified, they can be classified into 4 cases of well-differentiated type of tubular adenocarcinoma (tub-1), 5 cases of poorly differentiated adenocarcinoma (por), 1 case of papillary adenocarcinoma, and 2 cases of signet ring cell carcinoma. In all the collection of xenografting materials, the primary foci were employed, while 3 cases of metastatic foci from lymph nodes were also employed. As indicated in Table 1, positive tumor formation was noted in 4 cases of Case Nos. 1, 2, 11, and 12, all of which proved to be transplantable. Consequently, the rate of successful transplantation was 4/12 (33.3%). Histological taxonomy resulted in 2/4 of tub-1 (50%), 1/1 (100%) of pap, and 1/5 (20%) of por, thus showing a low rate of successful transplantation of 20% for poorly differentiated adenocarcinoma. The signet ring cell carcinoma could not be transplanted. Fig. 1 illustrates the results of determination of the volume doubling time of the third transplantation strain with (major axis \times minor axis)^{3/2} on the skin surface of the nude mice. The volume doubling time in each transplanted strain was 5 days in No. 1, 8 days in No. 2, 4 days in No. 11, 3 days in No. 12, and 4 days in No. 13. The poorly differentiated adenocarcinoma in No. 12 propagated fairly rapidly, but the two well-differentiated type of Nos. 2 and 11 demonstrated some differences between them, showing no fixed propagation rate on the histological patterns. The transplantation of subcutaneous tissues which served as the bases of our present experiment is illustrated in Fig. 2. The histological findings on 4 strains of transplantable human gastric cancer and 1 strain of canine experimental gastric cancer are illustrated in Figs. 3 to 7. Figs. 3(a) and
(b) illustrate the histological findings of the primary focus of No. 1 and the 21st transgrafting in the subcutaneous tissue of a nude mouse, identified as papillary adenocarcinoma. Figs. 4(a) and (b) illustrate the primary focus of Case No. 2 and the 10th transgrafting in the subcutaneous tissue of a nude mouse, identified as well-differentiated tubular adenocarcinoma. Figs. 5(a) and (b) illustrate the primary focus of Case No. 11 and the 5th transgrafting in the subcutaneous tissue of a nude mouse, identified as a well-differentiated tubular adenocarcinoma. Figs. 6(a) and (b) illustrate the primary focus of Case No. 12 and the 4th transgrafting in the subcutaneous tissue of a nude mouse, identified as a poorly differentiated adenocarcinoma. Figs. 7(a) and (b) illustrate the primary focus of Case No. 13 (canine experimental gastric cancer) and the 10th transgrafting in the subcutaneous tissue of a nude mouse, identified as well-differentiated tubular adenocarcinoma. As demonstrated in these findings, the histolo-
Fig. 4(a). Primary focus of No. 2 strain, well differentiated tubular adenocarcinoma. (H-E. ×200)

Fig. 4(b). 10th transfer of No. 2 strain. (H-E. ×200)

Fig. 5(a). Primary focus of case of No. 11, well differentiated tubular adenocarcinoma. (H-E. ×200)

Fig. 5(b). 5th transfer of No. 1 strain. (H-E. ×200)

Fig. 6(a). Primary focus of No. 12 strain, poorly differentiated adenocarcinoma. (H-E. ×200)

Fig. 6(b). 4th transfer of No. 4 strain. (H-E. ×200)

gical patterns of the primary focus were maintained extremely well during the repeated transplantation. No essential difference was found between the tumor in the recipient and the parent tumor. Grafted tumor had abundant cytoplasms, nuclei of variable sizes, and a high mitotic activity and mucin positivity, whenever initially present tumor was retained. In tumor-bearing mice, there was minimum inflammatory reaction consisting of a few mononucleated cells. The only difference between the ori-
gin and recipient was the reduced stroma and dense tubular structure at every passage, and this tendency was one of general characteristics in the experiment with nude mice.

2. Investigation on strains of exclusive uses for intraperitoneal transplantation

Intraperitoneal transplantation test was performed in nude mice by using strains Nos. 2 and 11. In the No. 2 strain, the 4th, 5th, and 6th transplanted strains of subcutaneous tissues were employed. Eight animals were effectively used for the experiment. In the No. 11 strain, the strain transplanted mainly from the metastatic lymph nodes of the primary focus was used. In this experiment, the 1st and 2nd transplanted strains of subcutaneous tissues were employed. Ten animals were effectively used for the experiment. The histological findings in both strains Nos. 2 and 11 will be described hereinafter.

(1) Histological evaluation in strain No. 2

No finding of infiltration into the muscular layer of abdominal wall was obtained in the tumor transplanted into the peritoneal cavity. In the cases with propagation in the form of small nodules through nidation
upon peritoneum, the findings were similar to those in the extensive miliary type of carcinomatous peritonitis, as shown in Fig. 8. In the findings of implantation and propagation on the digestive tracts, a trend was clearly noted that the transplanted tumor was propagated in a spherical form surrounded by digestive tracts, especially by mesenterium. In the cases, however, with implantation and propagation on the gastric wall, the image of infiltration directly on the gastric wall was noted. Fig. 9 illustrates the cases with implantation and propagation, in which the tumor was infiltrating from the side of gastric serosa toward the muscular layer, and a part of its extended as far as the submucosal layer. In this case, the transplanted tumor was found propagated as if it were extensively covering the serosa of esophageal phrenic ampulla. The same trend of infiltration was also found in the cases with pyloric implantation and infiltration. Fig. 10 illustrates the infiltration into the muscular layer of pylorus. In the cases with nidation and propagation on the mesenterium, tumors of varying sizes, well capsulated, were found scattered. In some cases, implantation on liver and pancreas was also noted, in which, however, signs of infiltration of cancer cells into parenchyma were not detected. No sign of infiltration into the intrapelvic parenchymal organs was found, but propagation into the intrapelvic peritoneum and fatty tissues was found.

(2) Histological evaluation in No. 11 strain

The transplanted tumor was implanted and propagated in the peritoneum after intraperitoneal transplantation, which proceeded only toward the muscular layer of the abdominal wall in an outwardly pushing manner, and no sign of infiltration was
found. The military propagation pattern in the peritoneum which was noted in the No. 2 strain also could not be found.

A sign of infiltration was seen in the cases with implantation and propagation in the digestive tracts. Fig. 11 illustrates the infiltration of cancer cells which had been implanted and propagated in the duodenum, from the side of serosa toward the muscular layer and mucosal layer. The cancer cells infiltrated among the muscular layer, reaching the submucosal and mucosal layer. In the upper mucosal layer of the same region, cancer cells of the transplanted tumor was found scattered, surrounded by endothelial cells, among the gland of duodenal mucosa, as illustrated in Fig. 12. As the nucleus of the endothelial cells was located close to the face of the inner lumen, cancer cells were judged as making the intralymphatic per-

mation into the mucosal layer. Hardly any interstitial reactions such as the cellular infiltration against the cancer cell infiltration were noted. Host infiltration by monocellular and polymorphonuclear cells was either absent or scanty. Fig. 15 illustrates the implantation and propagation on the wall of small intestine, in which it can be seen that cancer cells were found to have infiltrated into the submucosal layer, by damaging the muscular layer from the side of serosa of small intestine. In the case of implantation and propagation on the mesenterium, a trend was noted to grow in a bulbar form as with the No. 2 strain. Infiltration of the transplanted tumor into the intrapelvic organs was also found, which was not obtained with the No. 2 strain. Fig. 14 illustrates the implantation and propagation on the vesical wall, in which it
is noted that the cancer cells were found to have directly infiltrated into the vesical muscular layer. In the intrapelvic fatty tissues, a strong trend was noted for implantation and propagation of transplanted tumor.

3. Investigation in the group of experiment with mixed strains

Insofar as we could follow up, there was no report on the use of a mixture of multiple strains in the same nude mouse. In our present study, we performed the experiment with an aim to know the histological characteristics of various types of mixed strains, making the most of the multiply transplantable strain. The mixed strain composed of these multiply transplantable strains using the subcutaneous tissues was transplanted into the peritoneum of the nude mice. The unit amount for the intraperitoneal infusion was the same as the quantity of 8 mm$^3$ (with an edge of 2 mm) with mixing of each strain. The strain of the 21st or 22nd transplanted generation with subcutaneous tissue was employed for No. 1 strain, while the 5th or 6th transplanted generation was used for No. 11 strain. The 4th or 5th transplantable generation was used for the No. 12 strain, while the 10th one was employed for

Fig. 13. Direct invasion of cancer cells into the submucosal layer of the small intestine in No. 11 strain. (#454, H-E. ×100)

Fig. 14. Cancer cell invasion of muscle layer of urinary bladder in No. 11 strain. (#454, H-E. ×100)
the No. 13 strain. The test was performed with division into the two groups of one, the mixed group of 4 transplantable strains of human gastric cancer origin and the other, the mixed group with the above plus the No. 13 strain derived from the canine experimental gastric cancer. The effective animals were 4 each in the two groups, making 8 animals in total.

The transplanted tumor was more vital than that of the single strain and enlarging tumors were found scattered. Summary of gross finding revealed that the regions of implantation and propagation of the transplanted tumors were the peritoneum, walls of the digestive tract, capsules of greater and smaller omentum, mesenterium, and pelvic cavity, which were not very different from
those of the single strains. Fig. 15 illustrates one of the gross findings at autopsy. The transplanted tumor propagated in the form of conglomerate nodules in the abdomen in an aggregate. The entire tumor was well capsulated, and newly developed and dilated blood vessels derived from nude mice were identified as in the cases of single strains. The tumor was grayish white and was tightly adhered to the peritoneum, which was encapsulated, but had an extensive necrosis in its center. Especially to be noted was that the implantation and propagation were remarkable in the pelvic cavity, and the largest tumor was as large as a diameter of 20 mm. Grossly, neither metastasis into lymph nodes, lungs, or liver, nor ascites was found in any of the animals.

One of the histological characteristics noted with the mixed strain was that the
mixed strain which propagated as an aggregate within the peritoneum and pelvic cavity was found co-existing like a collided cancer. Especially to be noted was that each of tissue type could propagate independently at various sites without any intervention in the mouse, in either of the strain of canine experimental gastric cancer or human gastric cancer. For example, Fig. 16 illustrates the histological finding of the implantation and propagation on the mesenterium, in which the tissue patterns of Nos. 1, 11, 12, and 13 were mixed, without forming any clear-cut bulkhead between each. On the borderline of propagated heterogeneous tubular gland, a tubular gland canalculus formed a front with another one without developing any bulkhead. Fig. 17 illustrates the borderline between the No. 1 and No. 2 strains, where the tubular gland of each grew and formed a front as shown with an arrow, as if both could form a united tubular gland. The
same was noted also with the well differentiated and poorly differentiated tubular adenocarcinomas. Fig. 18 illustrates the formation of a front (indicated with an arrow) by the Nos. 2 and 12 strains. The interstitial reactions of mice against the transplanted mixed strain were extremely rare as with the single strain group.

The transplanted tumor implanted and propagated in the peritoneum, infiltrated continuously into the muscular layer of the abdominal wall as illustrated in Fig. 19, in which the histological patterns of strains Nos. 2 and 12 were noted, but without any clear-cut bulkhead between them.

As noted in the group of single strain, the pattern of infiltration of transplanted tumor directly from the wall of the digestive tract toward the inner cavity could not be found in the case of digestive tracts.

Fig. 20 illustrates the pattern of metastasis into liver, histologically found by accident in the liver parenchyma. This case was subjected to autopsy at day 30 of transplantation with 5 kinds of mixed strains, which would possibly be claimed to be the first case reported on its hematogenous metastasis of the strain of human gastric cancer into the liver of nude mice.

The metastatic focus in to liver agreed histologically with the poorly differentiated medullary adenocarcinoma of No. 12 strain. The serial sections of this region indicated that the metastatic focus was gradually and continually reduced. Implantation of the transplanted tumor on the surface of liver in the vicinity of the metastatic focus of liver was neither grossly nor histologically noted. Therefore, the particular metastasis was judged as hematogenous metastasis into the liver. The largest axis of the metastatic focus in the liver was 2 mm, and a necrotic focus was found in the center of the metastatic focus, as indicated in Fig. 21. The liver parenchyma in which the metastatic focus was noted was found highly damaged in coincidence with the finding on the infections by mouse hepatitis virus. Also, in the vicinity of the metastatic focus, necrotic foci of tissues of liver cells were found scattered, and also found in the tissues of liver cells under oppression and pushing by
the metastatic foci. In both the single and mixed groups, the transplanted tumor located on liver surface did only propagate on the surface of liver in an oppressed and pushed manner, and no finding of infiltration into parenchyma of liver could be noted.

To summarize the results of the above experiments, the following may be commented.

In the single strain groups: The tumor implanted in the peritoneum propagated in an oppressive and push-out manner. The tumor implanted on the wall of digestive tract tended to infiltrate directly into the mucosal layer from the serosa. On the mucosa, the lymphatic permeation of cancer cells was noted. The infiltration into the wall of the intrapelvic organ (urinary bladders) was also noted. The tumor also tended to propagate in a scattered manner in the fatty tissues in the pelvis.

In the mixed strain group: Each transplantable strain co-existed and propagated without intervention on the other. On the borderline between the heterogeneous strains, the cancer glands of these strains were found propagating, forming a front. The human gastric cancer strain and canine gastric cancer group were found to be propagating in the same region. In one of the human gastric strains, hematogenous metastasis into the liver was noted.

DISCUSSION

Since Rygaard and Povlsen\(^3\) reported the transplantation of human colonic cancer into nude mice in 1969, many oncological studies on nude mice bearing human malignant neoplasms have been published.

Although transplanted tumor grew well in nude mice, and morphological and functional characteristics were retained intact in tumor-bearing nude mice, the parent tumor was localized and encapsulated, showing no metastasis or invasion. Only malignant lymphoma (Burkitt) and malignant melanoma develop such malignant characteristics in nude mice.\(^2\)-\(^4\) Spontaneous tumor in nude mice has not been recognized so far except in a report on the reticulum cell, type B, of malignant lymphoma.\(^1\)

Why does grafted human carcinoma grow in nude mice in localized type with no metastasis or invasion? This is a matter of controversy. There are some possibilities that the time required for metastasis and invasion to become clinically or histologically distinct may be longer than that we allowed for growth in any one mouse in our study. This could be one of the reasons. As shown in this study, however, at least in peritoneal cavity, invasion and hematogenous metastasis into the liver were verified in the case of human gastric cancer, which is one of the most difficult transplantable cancers. Therefore, it is not too much to say that a study with intraperitoneal administration to mice has a better background for cancer transplantation than the subcutaneous one as generally accepted.

In 1975, Ueyama\(^5\) succeeded in transplantation of the human gastric cancer into nude mice for the first time. According to their report, 5 cases of gastric cancer from surgically resected specimens were transplanted successfully.\(^5\) In the same year, Kuga also succeeded in the take of serial strains from liver metastasis in autopsied cases.\(^6\) Kurihara and Taguchi independently succeeded in grafting experimental canine gastric cancer induced by ENNG and/or MNNG into nude mice.\(^7,\(^12\)

In 1976, many reports were published in Japan, especially with respect to gastric cancer transplanted in nude mice. Ohsawa succeeded in the take of a fluid cell line of ascites in abdominal cavity of nude mice.
The fluid cell line was a signet ring cell carcinoma, which showed no peritonitis carcinomatosa in nude mice. Hojo succeeded in the take of a strain which was transplanted from tissue cultured cell line to nude mice. This was histologically a poorly differentiated adenocarcinoma. Therefore, fluid cell line of ascites is able to grow in nude mice and also, tissue cultured cell line in vitro is able to be transplanted into the nude mice.

Since then, it remains as a problem to take the fluid ascites type of well differentiated adenocarcinoma. Suzuki reported that among many histological types of gastric cancer, moderately well differentiated tubular adenocarcinoma showed a good serial transplantation rate of 55.6% (5/9). In his report, papillary type failed to transplant, but in our present study, this type was transplanted successfully. According to many reports, overall successful serial transplantation rate is about 20% in human gastric cancer. In 1977, Schmidt also succeeded in the take of strains from surgical materials.

The intravenous injection of human malignant neoplasms produced no detectable tumors in nude mice. In this study, trypsinized human gastric cancer cells (6–8×10⁶) were injected into the spleen of 10 mice and the result was failure to detect any hematogeneous metastasis.

As mentioned above, this paper is a preliminary study on intraperitoneal graft of human gastric cancer in nude mice. Direct invasion, cancer permeation into lymphatic duct, and liver metastasis in mice-bearing tumor were verified and, also, mixed strain, even though canine strain was added to the human strains, grew well independently without interference, making front formation. The fact that both human and canine gastric cancer grew in a co-existent state may be the major contribution for gastric cancer research.

Acknowledgement

The author gratefully acknowledges the encouragement and generous advice of Professor Tadashige Murakami, 1st Department of Surgery, and also acknowledges Assistant Professor Nozomu Aoki, Department of Clinical Pathology, of this University. The author wishes to thank Dr. Akira Matuda and Dr. Osamu Yoshioka of Department of Pharmacological Research, Nippon Kayaku Co., who gave the author a chance to breed the nude mice. Thanks are also expressed to Mrs. Yoko Takagi and Mrs. Shihoko Yamaguchi for their technical advice.

References


