Intraperitoneal Transplantation of Ascitic Cancer Cells from a 7,12-dimethylbenz[a]anthracene (DMBA)-induced Ovarian Cancer

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Summary: Intra-abdominal implantation of 7,12 dimethylbenz[a]anthracene (DMBA)-induced rat ovarian cancer tissue produces intraperitoneal carcinomatosis with a high incidence. The peritoneal carcinoma produces malignant ascites in 62% of the donor rats. The ascites is bloody in appearance and includes an average of 1.2×10^6 cancer cells/ml. To observe the transplantability of the ascites, 0.1 ml of a condensed ascites with 4×10^6 cancer cells was injected into the abdominal cavity of 118 infant rats that were 2 to 4 days old. In 103 rats (87%), the ascitic cells were successfully transplanted. Twelve rats were sacrificed each week from the 2nd to the 6th week following the inoculation. The omentum was the first site at which the metastatic tumor appeared following the inoculation. Then the tumor disseminated throughout the intraperitoneal cavity and produced bloody ascites by the 3rd week. Eighty-four rats were observed to determine the survival, and it was 34 ± 10 days. Cytologically the ascites had clusters of tumor cells resembling bunches of grapes. The ultrastructure of the ascitic cells was globular shaped with many microvilli and epithelial attachments. The histology of the developed tumor was that of an adenocarcinoma. Due to morphological similarity with advanced human ovarian cancer and the high reproducibility, this experimental system could be a feasible model for human ovarian cancer, especially the type which produces malignant ascites.

Key words: DMBA-induced ovarian cancer — malignant ascites — experimental model — ascitic cancer — intraperitoneal carcinomatosis

Introduction

Since ovarian cancer is the most fatal gynecological malignancy, there is an urgent need for a reproducible experimental cancer that produces malignant ascites. Ascitic fluid in patients with ovarian cancer is widely regarded as one of the most important prognostic factors (FIGO, 1985), yet very little has been reported on the biologic behavior of the malignant ascites.

An experimental ovarian cancer (Kato

et al. 1974), induced by direct application of DMBA is of interest because of the massive ovarian tumor formation, the histology of the adenocarcinoma and the bloody ascites. To discern the malignant nature of the ascitic fluid found in the ovarian cancer, an experimental study was conducted using DMBA-induced ovarian cancer tissue. In this paper, the morphological features, transplantability and finally the malignant nature of the ascites in rats with DMBA-induced ovarian cancer will be described.

Materials and Methods

Animals

Neonatal female rats of the Wistar strain, weighing approximately 10 gm were used. The rats were bred in the animal experimental center of Kurume University for this experiment.

Tumor cells

The original autochthonous tumor was induced by the local application of DMBA to the ovary (clipping method) (Kato et al. 1974; Tsunawaki, 1977) of a Wistar strain rat which weighed approximately 180 gm. The ovarian cancer proved to originate from the ovarian surface epithelium (Nishida et al. 1986).

The induced ovarian tumor was removed aseptically and minced into fragments 2.0 mm in diameter. One fragment was inserted subcutaneously in the back of a neonatal female rat of the same strain. These tumors were serially transplanted every 3 weeks and maintained for more than 60 generations in our laboratory. Morphologically, the autochthonous DMBA-induced tumor was almost the same as an adenocarcinoma with both glandular and solid structures. After serial transplantation, the tumor histology had a much more homogeneous solid pattern and the connective tissue and inflammatory reaction decreased remarkably in comparison with the primary ovarian cancer (Sugiyama et al. 1978, 1986). Therefore, the transplanted tumor was suitable for further experimental studies, such as intraperitoneal transplantation. The subcutaneously serial transplanted tumor was again excised and minced into fragments of 2.0 mm in diameter, and one fragment was implanted into the peritoneal cavity of a neonatal female rat of the same strain under ether anesthesia according to the method of Sugiyama et al. (1990). Visible abdominal distention

due to copious ascites was noted at the 4th to the 5th week following the intraperitoneal transplantation. The ascites was bloody and contained 1.2×10^6 tumor cells/ml.

Method of Transplantation

The malignant ascites was centrifuged (1500 rpm for 5 min), and the buffy coat cells were suspended in a 0.9% sodium chloride solution. The first group of neonatal female rats was inoculated with $4 \times 10^6/0.1$ ml tumor cells using a 27-gauge needle inserted into the abdominal cavity. Four to five weeks later, abdominal distention due to malignant ascites was observed in the rats. Subsequently, the ascites cells were injected i.p. into other hosts using the procedure described above. This serial transplantation was periodically repeated in a total of 118 rats (Fig. 1). On the other hand, 12 rats in the second group were inoculated with $2 \times 10^5/0.1$ ml tumor cells.

Fig. 1. Method of transplantation.

General observations

- 1) The success rate of i.p. transplantations was observed in 118 rats (inoculated with 4×10^6 tumor cells) for 6 generations and 12 rats (inoculated with 2×10^5 tumor cells).
- 2) The survival period was recorded for 84 tumor bearing rats inoculated with 4×10^6 tumor cells.
- 3) Twelve tumor bearing rats inoculated with 4×10^6 tumor cells were sacrificed to examine the spreading of the cancer each week from the 2nd to the 6th week following the injection.
- 4) Seven tumor bearing rats inoculated with 4×10^6 tumor cells were used in other experiments.

Morphological Observations

- 1) For cytological studies, ascitic fluid was collected when the animals were sacrificed. The number of tumor cells was counted with an hemocytometer, and smears of ascites were rapidly dried and fixed with 70% ethanol and Bouin fluid. The staining methods used were Giemsa, periodic acid-Schiff (PAS) and Papanicolaou stains.
- 2) For histological studies, solid tumors were also removed. The tissue specimens were fixed with Bouin fluid and stained with hematoxylin and eosin (HE).
- 3) For electron microscopy, cell blocks of ascites were fixed in 2.5% from glutaraldehyde, postfixed with 1.0% osmium acid, dehydrated in graded ethanol, and embedded in Epon 812. Ultrathin sections were double-stained with uranium and lead.

Results

In the first group, abdominal distention due to retention of bloody ascites was noted at the 4th to 5th week following the inoculation (Fig. 2a). Gross ex-

amination of the peritoneal cavity at this time revealed diffuse studding of tumor deposits on the peritoneal surfaces, viscera and diaphragm (Fig. 2b). The sizes of the tumor implants were variable with deposits from 1 mm in diameter on the diaphragm to 10-30 mm on the omentum and bowel mesentery. Twelve rats were sacrificed each week from the 2nd to the 6th week following the inoculation. The omentum was the first location in which the metastatic tumor appeared following the inoculation, after which the tumor

Fig. 2. Rats sacrificed at the 4th week after transplantation.

- a) Bloody ascites was present beneath the peritoneum
- b) Bulky tumors, disseminated tumors and ascites were noted throughout the peritoneal cavity.

disseminated throughout the intraperitoneal cavity. It produced bloody ascites at the 3rd week, resembling stage III human ovarian cancer (FIGO) (Table 1).

In the first group, the transplantability rate of the i.p. inoculation with 4×10^6 tumor cells was 87% (103/118). The remaining 15 rats inoculated with 4×10^6 tumor cells were alive 90 days after the transplantation, with no detectable tumor cells in the peritoneal cavity. This transplantability rate was not much different for all the generations during the serial transplantations. In the second group, all the 12 rats inoculated with 2×10^5 tumor cells were alive 90 days after transplantation, and no detectable tumor cells were found in the peritoneal cavity.

All the tumor bearing rats died from extensive i.p. disease (massive ascites and peritoneal carcinomatosis) within 1 to 2 weeks after the abdominal distention had been noted. The survival period for the 84 tumor bearing rats was 34 ± 10 days (Fig. 3).

The ascites fluid was bloody in ap-

pearance and contained 1.6×10^6 tumor cells/ml. The total number of nucleated cells was 8.9×10^6 cells/ml. The cytological specimens included 18% with tumor cells, 42% with neutrophils, 31% with lymphocytes, 5% with histiocytes and 4% with mesothelial cells by Giemsa stain. Some tumor cells were in papillary clusters like bunches of grapes, and others floated free in the ascites. They had large fine reticular nuclei which varied con-

Fig. 3. Survival periods of 84 tumor-bearing rats.

TABLE 1.
Spread of cancer in 12 tumor bearing rats

	case	cancer spread intraperitoneal cavity	malignant ascites (ml)
2nd week	1	omentum	0
	2	omentum	0
	3	omentum	0
3rd week	1	omentum	0
	2	omentum, peritoneum (including diaphragm)	4
	3	omentum, peritoneum (including diaphragm)	3
	4	omentum, peritoneum, liver	2
4th week	1	omentum, peritoneum (including diaphragm and mesenterium)	5
	2	omentum, peritoneum, liver	4
5th week	1	omentum, peritoneum (including mesenterium)	9
6th week	1	omentum, peritoneum (including diaphragm and mesenterium)	14
	2	omentum, peritoneum (including diaphragm), uterus	18

siderably in size with the Papanicolaou stain (Fig. 4). Mucin was negative in the cytoplasm of the tumor cells with the PAS stain. Electron microscopically, the tumor cells in the ascites formed a globular shape and possessed many microvilli. Junctional complexes of the tumor cells and remarkable nuclear atypia were also investigated. Large nucleoli with a basket structure and condensed chromatin in the margin of the nuclei were observed. A large number of mitochondria were noted

in the cytoplasm (Fig. 5).

Histological examination of the omental tumor at the 2nd week revealed an adenocarcinoma with a solid structure among the copious stroma (Fig. 6a). At the 4th week, the omental tumor developed various histologic features including solid and glandular structures in the scant stroma (Fig. 6b). This histology indicated a similarity to a high grade human serous tumor.

Fig. 4. Clusters of neoplastic cells like bunches of grapes in the ascites. (Papanicolaou, original magnification $\times 100$).

Fig. 5. Electron micrograph of tumor cells in the ascites that were globular shaped with many microvilli ($\times 3360$). Inset: epithelial attachment ($\times 6250$).

a

b

Fig. 6. Tumors in the omentum.

- a) An adenocarcinoma with a solid structure in the copious stroma was noted at the 2nd week after transplantation (H.E., original magnification $\times 50$).
- b) A concomitant adenocarcinoma with a glandular and solid structure was observed at the 4th week after transplantation (H.E., original magnification $\times 50$).

Discussion

Despite current advances in the treatment of ovarian cancer including improved methods of surgical management and the introduction of new cytotoxic drugs, treatment results for the advanced disease have been disappointingly unchanged for decades. The size of the residual tumor following cytoreductive surgery and the presence of malignant ascites have received special attention as prognostic determinants (Redman et al. 1986). Although chemotherapy has a certain place in the control of ovarian cancer, difficulties remain in the assessment of the therapeutic effect at the histocytological level (Noda, 1988). To resolve these problems, an appropriate experimental model is required.

A variety of experimental models have been examined to study the characteristics of the human malignant disease, including the morphology, biologic behavior and, recently the chemosensitivity. In vivo, serially transplantable human neoplasms have been established in the subcutaneous tissue of nude mice (Goldin et al. 1980). In general, however, the slow growth of the human solid tumors and the prolonged survival of tumor bearing mice impeded the cancer chemosensitivity testing (Povlsen and Jacobsen, 1975). Recently, establishment of an intraperitoneal ascitic xenograft of human ovarian and breast cancer in nude mice was reported (Hirohashi et al. 1976; Hamilton et al. 1984). Although these models are useful for chemosensitivity testing of human cancer tissue, a problem concerning immunological relationships between tumors and hosts remains to be resolved. In this sense, an experimentally induced primary adenocarcinoma of the ovary (Kato et al. 1974) in the rat is unique and promising. The tumor tissue was surgically transplantable to the subcutis (Sugiyama et al. 1978, 1986) and

intraperitoneal cavity (Sugiyama et al. 1990) suggesting the feasibility of this experimental system for cancer chemosensitivity testing. However, presumably due to technical problems with the intraperitoneal transplantation, the reported transplantability rate was only 62% with this method (Sugiyama et al. 1990).

In the present study, a transplantability rate of 87% (inoculated with 4×10^6 tumor cells) was obtained, indicating that i.p. injection was more feasible than the surgical procedure. Using i.p. transplantation, experimental models for human ovarian cancer in advanced stages (Stage IIIa to IV, FIGO) were easily produced within a short period of 3 to 5 weeks. In addition, histological and cytological examinations showed the morphological similarities to human cancer.

This model is different from the so-called "ascites cancer", but is considered to be an experimental model for "carcinomatous peritonitis". The model has clear-cut parameters to evaluate the anticancer effects, including changes in the survival period of the host and alterations of the morphology of the tumor tissue and ascitic cancer cells. Using this model, more detailed observations on the mechanisms of anticancer agents will be possible.

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