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ORIGINAL ARTICLE

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Influence of surgical trauma on tumor establishment in a rat ovarian cancer model

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Abstract There is a strong debate regarding the influence of laparoscopic surgery on tumor dissemination and prognosis, especially in ovarian cancer. In murine mammary cancer cell line models, the incidence of surgical metastasis was influenced by the type of surgical trauma. We investigated the influence of surgical trauma on tumor establishment in a synergetic rat ovarian cancer model in 6- to 8-week-old female BD-IX rats using the rat ovarian cancer cell line O-342. The animals underwent i.p. injection of tumor cells, laparoscopy or midline laparotomy with different surgical procedures at the right uterine horn (grasping with a forceps, incision of the uterine horn after coagulation or partial resection of the uterine horn by ligation). Intraperitoneal tumor establishment was then determined 14 days after the surgical procedure, using cancer index scoring (0, no tumor; 1, tumor diameter less than 0.5 cm; 2, between 0.5 and <1.0 cm; 3, between 1.0 and <2.0 cm). No macroscopically visible tumor growth was observed in the uterine horn, which was unaffected by any surgical procedure. Alternatively, for the right uterine horn that underwent grasping by forceps, coagulation or ligation, the cancer index score was found to be 0.2, 2.5 and 3.0, respectively. The present study shows that surgical trauma favors the establishment of tumor lesions in the rat ovarian cancer model.

Keywords O-342 · BD-IX rat · Animal model · Laparoscopy

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Introduction

Laparoscopic surgery has become the gold standard in the operative treatment of many gynecologic diseases. Intense debate has arisen regarding the safety of laparoscopic surgery in cases of adnexal masses later found to be ovarian cancer. Definitive human clinical data are not available to address the influence of laparosopic surgery on tumor dissemination and prognosis in ovarian cancer [1].

The influence of pneumoperitoneum, gasless laparoscopy and other factors on the growth and dissemination of tumors has been investigated in many animal studies. Several animal studies performed with colon cancer or mammary cancer cell lines showed that the tumor growth was greater after laparotomy than after laparoscopy [2, 3, 4]. In the nude rat ovarian cancer model, CO_2 pneumoperitoneum had no detrimental effect on omental weight in comparison to laparotomy [5]. However, in a syngenic rat ovarian cancer model, Canis et al. [6] showed that tumor growth was greater after laparotomy than after laparoscopy. As in other studies with non-ovarian cancer cell lines, these authors found more severe tumor dissemination after laparoscopy compared to laparotomy.

Murthy et al. [7] reported that the incidence of surgical metastasis was influenced by the type of surgical trauma in the mouse model. Therefore, we investigated the influence of surgical trauma on tumor establishment in a syngenic rat ovarian cancer model in our study.

Material and methods

Animals

Six- to 8-week-old female BD-IX rats (Charles River, Sulzfeld, Germany) weighing 150–200 g were used for all experiments. The animals were kept under standard laboratory conditions (12 h light/12 h dark, room temperature 25°C, relative humidity 55%) with free access to standard laboratory diet and water ad libitum before and after the experimental procedure. The protocol was approved by a local committee on animal research.

Table 1 Experimental setup of the operative groups

Experimental group		Procedure: left uterine horn	Procedure: right uterine horn	I.p. injection of 10^3 ovarian cancer cells (O-342)
1	Laparoscopy	None	Incision of uterine horn after coagulation at three different locations in an area of 1 cm	Yes
2	Laparotomy	None	Grasping with a forceps	Yes
3	Laparotomy	None	Incision of uterine horn after coagulation at three different locations in an area of 1 cm	Yes
4	Laparotomy	None	Ligation of the right uterine horn combined with partial resection of uterine horn (1 cm)	Yes

Table 2 Cancer index scoring

Cancer index	Diameter of tumor nodules
0	No tumor
1	Diameter less than 0.5 cm
2	Diameter between 0.5 cm and <1.0 cm
3	Diameter between 1.0 cm and <2.0 cm
4	Diameter between 2.0 cm and <3.0 cm
5	Diameter exceeding 3 cm

Rat ovarian cancer cells

The epithelial ovarian cancer cell line O-342 (Cell Line Service, Heidelberg, Germany) was syngenic to BD-IX rats. The cells were cultured in DMEM with 10% fetal calf serum, 2 mmol glutamine and penicillin/streptomycin (100 IU/ml, 100 µg/ml) at 37°C in an atmosphere of 5% carbon dioxide. Cells were harvested after washing with phosphate buffered saline by trypsinating for 5 min. After three rounds of washing with phosphate buffered saline and centrifugation for 5 min at 1,000 rpm, the cells were resuspended in MEM with glutamine and without fetal calf serum.

Experimental procedures

The rats were anesthetized by intraperitoneal injection of ketamine (10 mg) and midazolam (0.5 mg).

Intraperitoneal injection of tumor cells

For determination of the lower limit of tumor cells that has to be applied for visible establishment of intraperitoneal tumor lesions, a different concentration of ovarian cancer cells O-342 was suspended in 1 ml MEM and was injected intraperitoneally. After 22 days, the animals were killed, and the presence of ascites and the size of the intraperitoneal tumor implants were registered.

Laparotomy

For laparotomy, the animal underwent a 3-cm midline incision. The right uterine horn was identified and the different procedures [grasping with a forceps, incision of the uterine horn after coagulation at three different locations in an area of 1 cm or ligation (vicryl 3.0; Ethicon, Norderstedt, Germany) of the right uterine combined with partial resection of the uterine horn (1 cm, Table 1)] carried out. After the procedure, tumor cell suspension was given in the lower abdomen. After 15 min, the abdominal wall was closed in two layers with vicryl 3.0. Fourteen days after laparotomy, the animals were killed, and the presence of ascites and size of the intraperitoneal tumor implants were scored with a semi-quantitative cancer score index (Table 2).

Laparoscopy

After incision of the skin, a laparoscopic sheath with an insufflation side port followed by a 4-mm arthroscope was inserted mid-abdominally. The pneumoperitoneum was maintained at 8 mmHg for 20 min. Two operating ports were implanted in the lower abdomen. After identification and coagulation and incision of the right uterine horn, the tumor cell suspension was injected into the lower abdomen by an operating port (Table 1). Skin incisions were closed with vicryl 3.0. Fourteen days after laparoscopy, the animals were killed. The presence of ascites and size of intraperitoneal tumor implants were scored with a semi-quantitative cancer score index (Table 2).

Results

No tumor growth or ascites development was observed macroscopically after the injection of 10^2 cells or 10^3 cells into the peritoneal cavity. By contrast, when more than 10^5 cells were injected, both ascites and intraperitoneal tumor growth were evident in all of the animals. After i.p. injection of 10^6 cells, two of three rats died, probably by intraperitoneal tumor growth and ascites. In these experimental group, 10 days after i.p. injection the development of ascites was obvious due to the blown up abdominal wall. Since surgical intervention was expected to promote tumor growth in the other study groups, the figure of 10^3 cells, in which no tumor growth was macroscopically evident, was selected for further experiments. Two of four animals in the group with 10^4 injected cells had already developed tumor implants (Table 3).

In the surgical groups, all of the animals were found to have tumor implants in the area of the abdominal wall scar, with the cancer index score being higher in the laparotomy groups than in the laparoscopy group, due to the larger area of the tumor implants. No macroscopically visible tumor growth in the omentum was noted in the laparoscopy group, while omental implants were found in all of the animals in the laparotomy groups (Table 4). Ascites developed in none of the animals in the laparoscopy group, in all of the animals that underwent laparotomy and coagulation or ligation of the uterine horn and in two of five animals in the group that received laparotomy combined with blunt trauma to the uterus.

The left uterine horn, in which no specific procedures were carried out in any of the animals, showed no visible tumor implants in any of the animals. By contrast, tumor implants were found in the right uterine horn, in which treatment was carried out to an extent dependent on the

Table 3 Injection of tumor cells dispersed in 1 ml MEM into abdominal cavity

Amount of injected cells	Number of animals	Number of animals alive at autopsy day 22	Number of animals with ascites	Number of animals with tumor in abdominal cavity
10^2	3	3/3	0/3	0/3
10^3	4	4/4	0/4	0/4
10^4	4	4/4	1/4	2/4
10^5	3	3/3	3/3	3/3
10^6	3	1/3	1/1	1/1

Table 4 Ascites and cancer index of major lesion at the uterus, omentum and adominal wall incision of the operative groups. 10^3 ovarian cancer cells (0–342) dispersed in 1 ml MEM were injected into abdomin immediately after manipulation at uterine horn. Animals were killed at day 14 after operative procedure. Group 1: laparoscopy and coagulation/incision of right uterine horn; group 2: laparotomy and grapsing of right uterine horn; group 3: laparotomy and coagulation/incision of right uterine horn; group 4: laparotomy and ligation/resection of right uterine horn

Group	Number of animals	Number of animals with acsites	Cancer index omentum (median)	Cancer index abdominal wall incision (median)	Cancer index left uterine horn (median)	Cancer index right uterine horn (median)
1	8	0/8	0	2	0	0
2	5	2/5	1	4	0	0.2
3	7	7/7	1	4	0	2.5
4	5	5/5	1	4	0	3

experimental group concerned. After blunt trauma, one of five animals had a macroscopically visible tumor implant; after laparotomy and coagulation/incision or ligation/resection of the right uterine horn, tumor growth was seen there in all of the animals.

Discussion

A large number of animal-model experiments have been carried out in the nude rat model to investigate the influence of laparoscopy on the spread and growth of ovarian carcinomas. As far as the present authors are aware, results have only been published once for experiments in the syngenetic rat model with BD IX rats, the same model used in the present study [6]. In in-vitro experiments, the SKOV3 ovarian carcinoma cell line was found to show increased growth under the influence of CO_2 [8]. By contrast, in various studies in which the nude rat model was used, neither the laparoscopy gas used nor laparoscopy itself was found to have any influence on various tumor parameters such as the weight of the omentum, metastases, survival of the animals or circulating tumor DNA, in comparison with laparotomy [5, 9, 10, 11, 12]. However, Canis et al. [6] observed increased tumor growth after laparotomy in the BD IX rat model in comparison with animals that underwent laparoscopic procedures. This is in agreement with the results of the present study. However, the increased tumor spread noted by Canis et al. [6] after laparoscopy was not observed. In our study, no ascites development and no macroscopic omental involvement were noted in the laparoscopy group, while they were observed in 14 of 17 cases, or in all animals, in the laparotomy groups. The higher cancer index with an abdominal wall incision in the laparotomy group, in comparison with the laparoscopy group, is explained by the primarily larger or longer incision in laparotomy. The increased tumor growth after laparotomy in comparison with laparoscopy, observed both by Canis et al. [6] and in our study in the rat ovarian cancer model, is confirmed by the findings of various research groups in the colon carcinoma model [2, 3, 4]. More marked postoperative immunosuppression after laparotomy might provide an explanation for this. In the murine flank tumor model, Da Costa et al. [13] showed that pulmonary metastases occur more frequently and that NK cytotoxicity is more strongly suppressed after laparotomy than after laparoscopy. On the other hand, Jacobi et al. [2] have shown that room air promotes intraperitoneal tumor growth. The greater tumor growth after laparotomy in comparison with laparoscopy might therefore be due to the effects of air.

Tumor growth in the uterus is dependent on the extent of surgical trauma. A higher cancer index can be observed in each uterine horn after coagulation or ligation than after blunt trauma. No macroscopic tumor growth was observed on the contralateral control uterine horn in which no surgical manipulation was carried out. This observation is in agreement with the results published by Murthy et al. [7], who showed that surgical trauma increases the probability of metastases developing after injection of a breast cancer cell line in the mouse model. Local factors appear to play a role in the development of metastases. However, despite coagulation, no macroscopic tumor development in the uterus was observed in the laparoscopy group. The tumor-promoting effects of surgical trauma appear to be less relative to the favorable effects of laparoscopy on tumor growth, in comparison with laparotomy.

Volz et al. [14] and Koster et al. [15] conclude from their studies of melanoma cells that pneumoperitoneum favors intraperitoneal tumor development. However,

these studies unfortunately did not include control groups in which open surgery was carried out. Their results can therefore not be used to answer the question of whether the open or endoscopic surgical access route is better. Despite the relatively small experimental groups used, the present study shows that surgical trauma favors the establishment of tumor lesions in rat ovarian cancer models.

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