Effect of Irradiation on Colony-Forming Ability of Stem Cells from Patients with Ovarian Cancer

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In patients with ovarian cancer, the colony-forming capacity and radiosensitivity of clonogenic tumor cells from the primary node and metastases (ascites) differed considerably.

Key Words: *clonogenic tumor cells; ovarian adenocarcinoma; radiosensitivity; primary and metastatic foci*

Studies of colony-forming clonogenic tumor cells are of considerable importance. These clonogenic cells belong to stem cells responsible for tumor growth, reactions to adverse factors, and metastasising [1]. *In vitro* cloning of tumor cells allows us to study human malignant tumors at the level of stem cells [8]. Colony formation in the system containing no colony-stimulating factors is the sign of carcinogenicity (malignancy). The content of clonogenic cells varies considerably in different tumors and individuals. Cloning of tumor cells is widely used for evaluation of tumor sensitivity to various chemical agents. Little is known about radiosensitivity of tumor cells.

Our previous studies revealed the possibility of cloning tumor cells from experimental animals and patients with various malignant neoplasms under modified conditions [2,3]. Human tumor cells are characterized by a lower cloning efficiency (CE) than tumor cells from experimental animals. The increase in CE is a prognostically unfavorable sign [2].

Here we studied the content of clonogenic cells in primary and metastatic foci in various patients with ovarian cancer (OC) and the reaction of these cells to damaging factors (radiosensitivity). Our studies are important for understanding of the mechanisms of radioresistance ovarian cancer, since the role of radiotherapy in the treatment of patients with these tumors remains unclear [6,10].

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MATERIALS AND METHODS

Tumor cells were isolated from surgically removed tissues from patients with OC (n=15) and ascitic fluid (n=21). We examined 32 patients. Only in 4 patients, the number of tumor cells isolated from primary and metastatic foci was sufficient for culturing. Single cell suspension was prepared by mechanical and enzymatic disintegration under sterile conditions. During mechanical disintegration, tumor samples were washed with medium 199 containing antibiotics (100 U or 100 mg penicillin and streptomycin per 1 ml medium), minced with scissors into small fragments (1-2 mm), suspended in fresh medium 199, and washed out using a syringe with needles with decreasing diameters. The suspension was filtered through a 4-layer nylon filter, and the cells were washed 2 times by centrifugation at 1000 rpm for 5-10 min. The supernatant was removed, and the precipitate was resuspended in a fresh portion of medium 199. Viable cells were counted after 0.2% trypan blue staining. During enzymatic disintegration, washed and minced tissue samples were incubated with 5 ml 0.25% trypsin containing 0.2 ml DNase (1 mg/ml) and/or collagenase (1 mg/ml) at 37°C for 20-30 min and then washed with medium 199 containing 1% fetal bovine or human serum (group AB) for enzyme inactivation. The suspension was pre-

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pared as described above. Since the efficiency of enzymatic disintegration was similar to that of mechanical disintegration, in our experiments we used mechanical disintegration. Ascitic fluid cells were washed 2 times by centrifugation in medium 199 at 1000 rpm for 5-10 min. The supernatant was removed, and the precipitate was resuspended in a fresh medium. Viable (trypan blue negative) cells were counted.

Tumor cells were cloned using a 2-layer agar system of Hamburger and Salmon with modifications [4]. The nutrient medium contained McCoy 5A medium (Flow or Serva), additives used for cloning of hemopoietic cells [9], 15% inactivated fetal bovine or human serum (group AB), and 10% agar (initial concentration 5 or 3.2%). The final concentrations of agar in the lower (bottom) and upper layers were 0.5 and 0.32%, respectively. To prepare the mixture, 10 ml tridistilled water was added to 500 or 320 mg agar. Agar was sterilized by boiling in a water bath for 1-2 min. McCoy 5A medium and fetal bovine serum were placed in penicillin flasks and heated in a water bath to 40-42°C. The solution was mixed with 10% boiling agar and stored at the same temperature. The nutrient medium (2 ml) with a final agar concentration of 0.5% (lower bottom layer) was transferred to 30-mm Petri dishes and incubated at room temperature for 15-20 min, and then the upper layer of agar nutrient medium (final agar concentration 0.32%) containing test cells in desired concentrations was layered. The dishes were incubated in an incubator (7.5% CO_2 and 92.5% air) for 10-14-days at 37°C. Colonies containing more than 50 cells were counted under a microscope (×70).

Radiosensitivity of tumor cells from patients with OC was studied *in vitro*. Cell suspension was placed in glass vials (0.5-1.0 ml) and subjected to ^{60}Co γ-irradiation in a dose rate of 1 Gy/min (AGAT apparatus). Before and during irradiation tumor cells were kept at a melting ice temperature. Control and irradiated cells were layered on the upper layer of the agar system. The survival of irradiated cells (compared to nonirradiated control cells) was estimated by the number of colonies. Parameters of the dose-survival curve were calculated by the least square method [5].

RESULTS

OC cells form discrete colonies, which can be easily counted.

The efficiency of colony formation in tumor cells obtained from surgically removed tissues varied from 1.8 to 565.0 colonies (mean 77.0±43.4 colonies/10^5 cultured viable tumor cells).

Our results are consistent with previous data on colony-forming capacity of human tumor cells from other organs [5,7]. Tumor cells from ascitic fluid were characterized by a higher efficiency of colony formation compared to cells from the primary tumor node (from 3.7 to 5000.0 colonies, mean 518.0±299.8 colonies/10^5 cultured tumor cells).

Our results indicate that the content of clonogenic tumor cells in metastases is much higher than in the primary node, which reflects more intensive tumor progression in metastatic sites. It should be emphasized that cells from the primary tumor node are more sensitive to dissociation procedure than cells from ascitic fluid, which partially determines different CE of these cell populations.

Since CE of irradiated cells is relatively low, a considerable number of tumor cells is required for evaluation of their radiosensitivity. Preparation of the suspension of tumor cells from the primary and metastatic foci of the same patient and studies of cell sur-

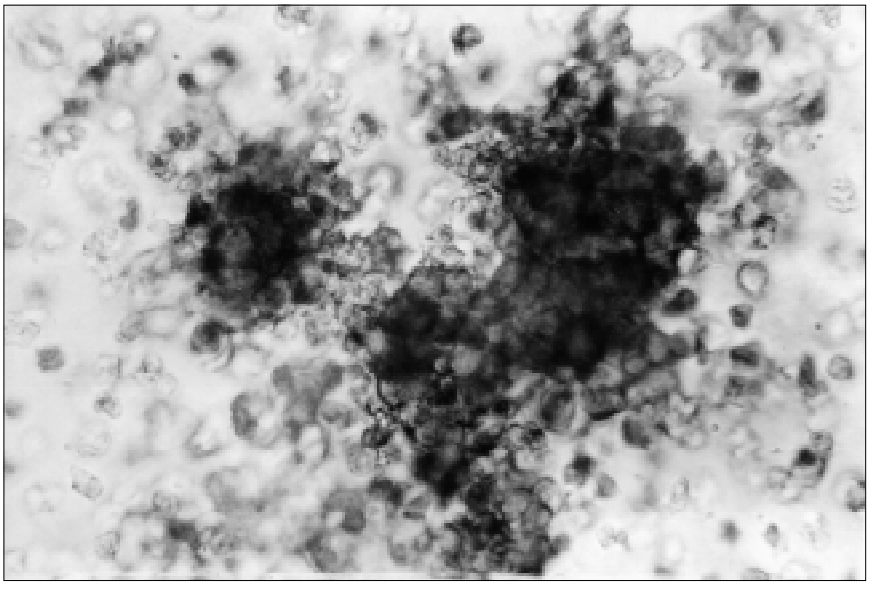

Fig. 1. Colonies of ovarian cancer cells (adenocarcinoma) in 14-day-old primary culture. Native preparation (×220).

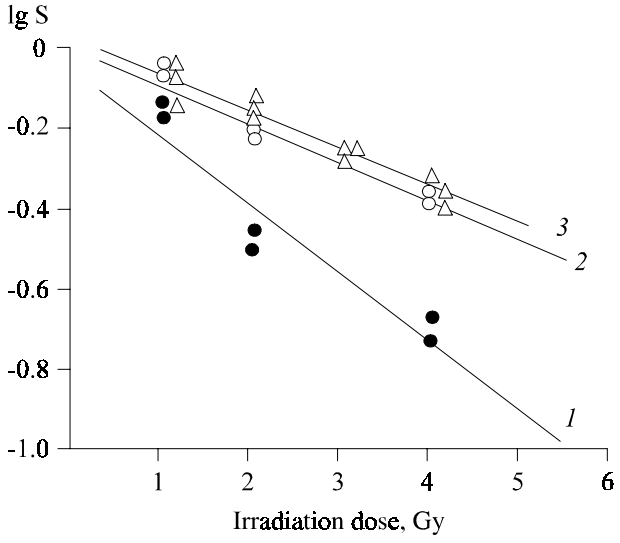

Fig. 2. Dose-dependent curve reflecting survival of clonogenic tumor cells from patients with ovarian cancer *in vitro* subjected to ^{60}Co γ-irradiation: tumor tissue from primary node in patient 1 (*1*, regression equation: lgS=-0.06-0.1679D); ascitic fluid from patient 1 (*2*, regression equation: lgS=0.07-0.0996D); and ascitic fluid from patient 2 (*3*, regression equation: lgS=0.0-0.085D).

vival after ^{60}Co γ-irradiation were possible only in 1 sample. Otherwise, the dose-survival curve was constructed only for ascitic fluid (Fig. 2). OC cells from the primary site were characterized by a higher radiosensitivity than cells from ascitic fluid. The mean lethal dose (D_0) and extrapolation number (*n*) for the primary tumor site were 2.59 Gy and 0.87, respectively; for cells from ascitic fluid these parameters were 4.36 Gy and 1.87 (patient 1) or 5.11 Gy and 1.0 (patient 2), respectively.

Thus, we revealed considerable differences in colony formation in primary clonal cultures and radiosensitivity of OC cells from the primary and metastatic foci. These differences probably reflect various stages of tumor cell progression and indicate relatively high resistance of OC (particularly, metastatic cells) to γ-irradiation. Our data on the resistance of clonogenic OC cells explanted into culture from the primary tumor site to γ-irradiation are consistent with previous examinations of 16 patients with papillary and serous ovarian adenocarcinoma [10]. In these patients, D_0 varied from 1.05 to 2.4 Gy (mean 1.7 Gy), *i.e.* was lower than in our experiments. It should be emphasized that these experiments were performed with early cell passages, but not with primary clonal cultures. This approach allowed to evaluate the sensitivity of clonogenic ovarian cells to γ-irradiation in 16 patients. Undoubtedly, primary clonal cultures of various tumor cells (including OC cells) are convenient models for studying the mechanisms of radioresistance and the effects of radiomodifying factors. However, these cultures cannot be widely used because of low CE. Elaboration of new methods of increasing colony-forming ability of tumor cells and obtaining early passages of cells is of considerable importance.

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