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Presence of both Mesenchymal and Carcinomatous Features in an In-vitro Model of Ovarian Carcinosarcoma Derived from Patients' Ascitic Fluid

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ABSTRACT

We have refined the technique for isolating and propagating cultures of primary ovarian carcinosarcoma cells (OSCs) derived from ascites, which allowed the cells to obtain the biphasic features of carcinosarcoma in cell culture conditions (presence of both carcinoma and mesenchymal morphologic types). This protocol involves a simple yet rapid method for the growth and propagation of ascites OSC in a basal culture medium. Autologous ascitic fluid was used as source of growth factors, and minimal manipulation was involved to establish the culture. The methodology allowed for the direct application of multiple molecular, cellular, and functional analyses within a few weeks of initial cell isolation, with the further potential of retrospective analyses of archived cells and tissues.

INTRODUCTION

Ovarian cancer constitutes nearly 4% of all cancers among women and is the leading cause of death from gynecologic malignancies in the western world. Among the various types of ovarian cancer, ovarian carcinosarcoma (OCS), also known as malignant mixed mullerian tumors (MMMT), remains one of the most rare and ill-defined histological subtypes[1]. Accounting for approximately only 1-4% of ovarian cancers, OCS carries a very grim prognosis due to the poor ability to study its rare histology and the lack of randomized clinical trials available to aid in the optimization of treatment strategies. Carcinosarcomas are known for containing dual histological elements including both carcinomatous (malignant epithelial) and sarcomatous (mesenchymal) elements.[1]The true genetic origin of OCS is unclear and several proposed theories have been put forth for debate. Three main theories have been proposed in this regard: First is the notion of a two cell collision theory, which postulates that two separate tumor types, epithelial and sarcoma are derived independently from each other, eventually colliding to form the carcinosarcoma. Second is the monoclonal cell theory, which proposes that the combination of sarcoma and carcinoma elements arise from one common stem cell precursor which may have undergone a divergent differentiation early in tumor development. The third theory is known as the conversion theory which suggests that one original cell type differentiates into a second cell type[2,3]. Given the notion that sarcomatous elements may be derived from carcinoma cell

precursors, our current mode of treatment used for OCS includes paclitaxel and platinum-based agents as used for other epithelial ovarian cancers.[1, 4, 5] However, as previously stated, our true understanding of optimal treatment for OCS is limited due to lack of randomized control drug trials secondary to the extreme rarity of this tumor.

Primary cell lines are valuable in vitro models for both clinical and basic studies. Among the numerous ovarian cancer cell lines, majority was generated from serous cystadenocarcinomas or poorly differentiated adenocarcinomas.[6,7,8,9] However, only a limited number of reports has shown the generation and propagation of ovarian carcinosarcoma primary cell lines.[10, 11, 12] In this study, we report a straightforward and simple protocol for the generation of an ovarian carcinosarcoma cell line from patients' ascitic fluid.

To the best of our knowledge, this is the first known primary OCS cell line reported to be developed from ascetic fluid.

MATERIALS AND METHODS

Isolation of primary ovarian cancer cells from ascites

Primary ovarian cancer cells were isolated from ascites specimens from 25 patients with ovarian cancer at the time of surgery or clinically indicated paracentesis. The study protocol was approved by the Institutional Review Board of Danbury Hospital. Approximately 250 mL of ascites was obtained from each patient. In each cell culture flask 20 ml of fresh ascites fluid was mixed with 20 ml of RPMI1640 media, supplemented with 10% FBS, 100 units/ml penicillin and 100 µg/ml streptomycin. Regular passaging of the cell lines was carried out in complete culture medium conditioned with 20% of their own medium and 10% of autologous filtered ascites. After 4–5 passages, cell cultures were completely free of fibroblasts and mesothelial cells. Aliquots of cells from all 25 patients in the study cohort were frozen in liquid nitrogen, at different passage numbers, in 5% dimethyl sulfoxide (DMSO) in FCS for future studies. Cells were used for phenotypic characterization after three days of culture (passage 1-2) and were fully confluent after six days of culture. Initial isolation of OSCs can be completed within 1 h, and primary cells are further expanded in culture for several weeks.

Immunofluorescence analysis of tumor cell epithelial origin

Primary cells isolated from ascites fluid were analyzed to assess the epithelial origin. A total of 1×10^6 cells were washed with PBS and non-specific binding was blocked with 5% BSA in PBS for 1 h at 37°C. Cells were washed with PBS and incubated for 20 minutes on ice in the dark with anti-cytokeratin mAb, followed by incubation with FITC-labeled secondary anti-mouse antibody. The cells were washed with PBS with 2% fetal calf serum (Sigma-Aldrich, St. Louis, MO) and cytospun into glass slides and analyzed with a microscope.

RESULTS AND DISCUSSION

Three days after establishment of the ascites fluid cell culture, specific sets of clones began to appear in the flask. Immunofluorescence studies confirmed the epithelial origin of the cells (data not shown). Among all cell lines we generated from ascites of ovarian cancer patients, the cell line shown in Fig. 1 resembled the distinct morphologic pattern and appearance seen in ovarian carcinosarcoma from solid tumor pathology. This cell line was from a 65 year old female with Stage 3c ovarian cancer, who was optimally debulked, and then received six cycles of carboplatin+taxol. Unfortunately a recurrence was observed within five months post adjuvant chemotherapy, thus patient was designated as platinum resistant. She then received additional cycles of chemotherapy by Doxil (Doxorubicin Hcl liposome injection) and then unfortunately died as a result of the disease progression. Anti-Cytokeratin staining confirmed the purity of primary epithelial ovarian cancer cell (data not shown). Morphologically, solid tumor OCS presents with two populations of cells: carcinomatous (malignant epithelial) and sarcomatous (mesenchymal) elements [1]. As figure-1 shows, the SS9 cell line contains both of these cell elements and thus represents a unique cell line derived from the ascetic fluid. The rounded cells in figure-1 represent the carcinomatous cells and the spindle shaped cells are the mesenchymal cells

which are both expected to be present in the native solid tumor of carcinosarcoma. According to the conversion theory for explaining the biphasic nature of carcinosarcoma, the observed phenomenon might represent conversion of carcinomatous (malignant epithelial) to sarcomatous (mesenchymal) cell types in-vitro, i.e. in-vitro metaplasia [1].

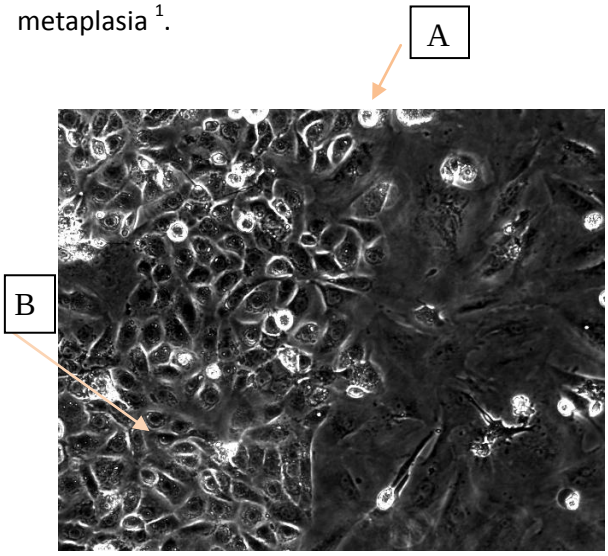

Figure-1: Microscopic image of SS9 cell line, with characteristic morphology similar to carcinoscahoma cell line in solid tumors. Two populations of cells can be identified here: A (Carcinomatous) and B (Sarcomatous elements)

Only a limited number of studies reported the generation and development of ex vivo primary cell lines from the solid tumor ovarian carcinosarcoma. Mobus et al. established the OV-MZ-22 cell line, derived from a solid tumor mass of ovarian carcinosarcoma. This cell line was aneuploid and showed no expression of the tumor-associated antigens CA-125 and CEA. It showed expression of MDR-1, lung resistance protein, p53, and topoisomerase I and II, but not of multidrug-resistance associated protein. The cell line also did not give rise to transplant tumors in nude mice.[12] Ide et al. reported establishment of another cell line from solid tumor ovarian carcinosarcoma mass that could grow without interruption for over 18 months, resulting in 43 passages after the initiation of the primary culture[10] .It showed pleomorphic and neoplastic features, the chromosomes of all metaphase plates show a human karyotype, as well as a wide aneuploidic distribution. This cell line expressed tumor markers such as CEA, CA-19*9 and CA125. To our knowledge, our study is first to report that OSC cells established from ascetic fluid can acquire the exact morphology of the original solid tumor while in vitro culture.

The protocol we reported in this study presents several advantages. Although other authors reported functional protocols for establishment of ovarian cancer cell lines from ascitic fluids,[6, 7, 8] these protocols involve more than 10 steps for cell culture establishment as well as extensive manipulation. These treatments might compromise the nature of the cell line. The protocol described herein doesn't present any enzymatic or mechanical manipulations thus preserving the original properties and physical features of the tumor cells. Additionally, the use of autologous ascetic fluid as growth factor allows the cells to maintain their native morphology and physiological features.

In conclusion, an efficient protocol to isolate and develop primary ovarian carcinosarcoma cell lines from patients' ascitic fluid was established in vitro. This may constitute the first known primary OCS cell line from ascetic fluid that presents the actual biphasic solid tumor morphology. This is of clinical importance as establishing primary OCS cell lines provides a powerful tool for further characterization of this rare tumor and also designing translation studies.

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