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In vivo tumor growth of high-grade serous ovarian cancer cell lines

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HIGHLIGHTS

- Eleven human cell models of high-grade serous ovarian cancer were tested in vivo tumor formation.
- OVCAR3, OVCAR5, and OVCAR8 were the most aggressive and OVCAR8 formed ascites.
- All six models formed peritoneal disease mimicking human cancer expressing p53, Pax8, and WT1.

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ABSTRACT

Objective. Genomic studies of ovarian cancer (OC) cell lines frequently used in research revealed that these cells do not fully represent high-grade serous ovarian cancer (HGSOC), the most common OC histologic type. However, OC lines that appear to genomically resemble HGSOC have not been extensively used and their growth characteristics in murine xenografts are essentially unknown.

Methods. To better understand growth patterns and characteristics of HGSOC cell lines in vivo, CAOV3, COV362, KURAMOCHI, NIH–OVCAR3, OVCAR4, OVCAR5, OVCAR8, OVSAHO, OVKATE, SNU119 and UWB1.289 cells were assessed for tumor formation in nude mice. Cells were injected intraperitoneally (i.p.) or subcutaneously (s.c.) in female athymic nude mice and allowed to grow (maximum of 90 days) and tumor formation was analyzed. All tumors were sectioned and assessed using H&E staining and immunohistochemistry for p53, PAX8 and WT1 expression.

Results. Six lines (OVCAR3, OVCAR4, OVCAR5, OVCAR8, CAOV3, and OVSAHO) formed i.p xenografts with HGSOC histology. OVKATE and COV362 formed s.c. tumors only. Rapid tumor formation was observed for OVCAR3, OVCAR5 and OVCAR8, but only OVCAR8 reliably formed ascites. Tumors derived from OVCAR3, OVCAR4, and OVKATE displayed papillary features. Of the 11 lines examined, three (Kuramochi, SNU119 and UWB1.289) were non-tumorigenic.

Conclusions. Our findings help further define which HGSOC cell models reliably generate tumors and/or ascites, critical information for preclinical drug development, validating in vitro findings, imaging and prevention studies by the OC research community.

1. Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer-related deaths among women in the US and the most lethal gynecologic malignancy [1]. The five-year survival rate has remained close to 25%, and all

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women are currently treated with the same approach consisting of surgical debulking followed by chemotherapy composed of paclitaxel and carboplatin [2]. Diagnosis of OC usually occurs after metastasis at stages II–IV, and this contributes to the poor survival [3]. Targeted therapies and better strategies for early detection would increase survival, but adequate model systems to study the disease remain a major challenge facing the gynecologic oncology research field [4,5].

Ovarian cancer is a heterogeneous disease that includes at least five histotypes: clear cell, endometrioid, mucinous, low-grade serous, and high-grade serous tumors [6,7]. Heterogeneity may be a result of the cell of origin that gives rise to different forms of the disease and reflects distinct molecular alterations associated with each histotype [8–10]. High-grade serous ovarian cancer (HGSOC), the most common and deadly form of the disease, is considered the "prototype" of epithelial OC, and the recent Cancer Genome Atlas Network analysis defined the landscape of deregulated pathways characterizing HGSOC [11]. Specifically, these tumors are classified based upon mutation of p53, BRCA1/2 mutation, somatic loss, or methylation, and a variety of protein markers including PAX8 and WT1. In addition, copy number variation is a hallmark of HGSOC and less commonly found in endometrioid, clear cell, and mucinous histotypes [12]. Recent genetic signatures from primary human tumors further divided HGSOC into four molecular groups, namely immunoreactive, proliferative, differentiated, and mesenchymal [13]. While these categories are well established in primary and recurrent HGSOC tumors, the ability to correlate genomic and molecular features with useful laboratory model systems is critical for the future development of new therapies, prevention strategies, and imaging studies [14].

Recent publications have characterized an expanded panel of OC cell lines at the genomic level, in 2-dimensional-cell culture (on plastic), and in regard to their in vitro response to chemotherapeutic drugs [15–17]. These reports further suggested that OC cell lines commonly used in the past (e.g., SKOV3, A2780) do not represent a good approximation of the HGSOC genotype and that a panel of recently described cell lines more closely resemble human serous tumor. However, several of the newly proposed models for HGSOC have never been characterized for the ability to form tumors in immune deficient mice, which is critical to study mechanisms of disease or therapeutic interventions in vivo. The goal of this study was to determine the tumorigenic ability of newly described HGSOC cell lines and the histologic characteristics of the xenografts derived from these cells.

2. Materials and methods

2.1. Cell culture

All reagents were obtained from Life Technologies (Carlsbad, CA) unless otherwise indicated. OVCAR4 was obtained through Material Transfer Agreement (MTA) from the National Cancer Institute for the transfer of cell lines from the Division of Cancer Treatment and Diagnosis Tumor Repository. The DCTD Tumor Repository has maintained, since the early 1960s, a low temperature repository of transplantable tumor and tumor cell lines from various species. OVCAR4 were maintained in RPMI supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 U/ml penicillin/streptomycin. Kuramochi, OVSAHO, and OVKATE were obtained through MTA from the Japanese Collection of Research Bioresources Cell Bank (JCRB). The JCRB cells were cultured in RPMI 1640 medium with 10% FBS. NIH:OVCAR3, CAOV3 and UWB1.289 cells were purchased from ATCC (1/2014). NIH:OVCAR3 cells were maintained In RPMI-1640 Media supplemented with 20% FBS, 0.01 mg/ml insulin and 50 U/mL penicillin, and 50 μg/mL streptomycin. CAOV3 cells were grown in Dulbecco's Modified Eagles Medium containing 10% FBS and 50 U/mL penicillin, and 50 μg/mL streptomycin. OVCAR5 cells were obtained from the Developmental Therapeutics Program at National Cancer Institute and cultured in DMEM, 10%FBS, 1% PSG, and 0.1 mM MEM Non-essential amino acids. OVCAR8 cells were obtained from ATCC and cultured in DMEM with 10% FBS. COV362 were from Adam Karpf, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center and grown in DMEM with L-glutamine (300 mg/L) and 10% heat inactivated fetal bovine serum. SNU-119 were sourced from the Korean Cell Line Bank (also obtained from Dr. Karpf) and grown in RPMI1640 with L-glutamine (300 mg/L), 25 mM HEPES and 25 mM NaHCO3, 90%; heat inactivated fetal bovine serum (FBS), 10%. UWB1.289 cells were cultured in media composed of 1:1 RPMI-1640 and Mammary Epithelial Growth Medium (MEGM, Lonza #CC-3150) supplemented by 3% FBS. Information regarding mycoplasma testing, in vitro doubling times, and STR validation is in Supplemental Table 1.

2.2. Study approval

All animals were treated in accordance with the NIH guidelines for Laboratory Animals and established Institutional Animal Use and Care protocols at the University of Illinois at Chicago, Indiana University School of Medicine, Indiana University, Bloomington, and University of Notre Dame.

2.3. Xenografting

6–7 weeks old female athymic (nude) mice were acquired from (Harlan Teklad, Indianapolis, IN) and xenografted with human OC cells 1×10^6 cells subcutaneously (s.c.) and 5×10^6 cells intraperitoneally (i.p.) in sterile PBS. Animal body weight and s.c. tumor growth (via caliper measurement) were tracked weekly and animals sacrificed when tumor burden was evident or general health was determined to be moribund. If no tumor formation was evident, animals were sacrificed after 90 days of tumor implantation.

2.4. Tissue collection and analysis

At the time of sacrifice, s.c. and i.p. tumors were dissected and weighed, and evidence of i.p. disease was noted by photography and charted based on organ of dissemination. Tissues were fixed in 4% paraformaldehyde before dehydration in ethanol and xylene prior to paraffin embedding. Immunohistochemistry and hematoxylin and eosin (H&E) staining was performed as previously described [18]. Briefly, tissues were sectioned and rehydrated in a gradient of ethanol prior to antigen retrieval and peroxidase block. Sections were incubated in primary antibody overnight at 4 °C before detection via biotinylated secondary antibody (1:200, Vector Laboratories (Burlingame, CA) and ABC peroxidase (Vector Laboratories)). Targets were visualized via 3,3′-diaminobenzidine (DAB, Vector Laboratories) and counterstained with hematoxylin. The following antibodies were used in the study: p53 (Santa Cruz Biotechnology sc-6243 dilution 1:50), Pax8 (Proteintech 10336-1-AP dilution 1:150), and WT1 (Abcam ab89901 dilution 1:50).

3. Results

To assess which HGSOC cell lines recapitulate OC clinical features in vivo, xenograft assays and pathologic characterization of resulting tumors were performed. Kuramochi, OVSAHO, SNU118, COV362, and OVCAR4 were the top five most likely to be high-grade serous ovarian cancer according to the genomic data analysis published by Domcke et al. [17]. The same report identified CAOV3, OVCAR3, and OVCAR8 as possible representatives of high-grade serous cancer. Additionally, CAOV3, Kuramochi, OVCAR3, OVCAR4, OVCAR5, and OVCAR8 were identified as high grade serous by Anglesio et al. [15]. UWB1.289 was chosen because it is BRCA-null [19]. Eleven OC cell lines were injected i.p. and/or s.c. into female nu/nu mice and tumor formation was assessed after observation (up to 90 days). Six of the cell lines (OVCAR3, OVCAR4, OVCAR5, OVCAR8, CAOV3, and OVSAHO) formed i.p xenografts (Fig. 1, Table 1) and were considered tumorigenic. OVKATE and COV362 only