NEOPLASIA
www.neoplasia.com

Volume 19 Number xx Month 2017 pp. 1–9 1

Prevention of Human Lymphoproliferative Tumor Formation in Ovarian Cancer Patient-Derived Xenografts[1,2]

Kristina A. Butler[*], Xiaonan Hou[†], Marc A. Becker[†,3], Valentina Zanfagnin[†], Sergio Enderica-Gonzalez[†], Daniel Visscher[‡], Kimberly R. Kalli[†], Piyawan Tienchaianada[†], Paul Haluska[†,4] and S. John Weroha[†]

[*]Department of Gynecologic Surgery, Mayo Clinic, Rochester, MN, 55905; [†]Department of Medical Oncology, Mayo Clinic, Rochester, MN, 55905; [‡]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, 55905

Abstract

Interest in preclinical drug development for ovarian cancer has stimulated development of patient-derived xenograft (PDX) or tumorgraft models. However, the unintended formation of human lymphoma in severe combined immunodeficiency (SCID) mice from Epstein-Barr virus (EBV)–infected human lymphocytes can be problematic. In this study, we have characterized ovarian cancer PDXs which developed human lymphomas and explore methods to suppress lymphoproliferative growth. Fresh human ovarian tumors from 568 patients were transplanted intraperitoneally in SCID mice. A subset of PDX models demonstrated atypical patterns of dissemination with mediastinal masses, hepatosplenomegaly, and CD45-positive lymphoblastic atypia without ovarian tumor engraftment. Expression of human CD20 but not CD3 supported a B-cell lineage, and EBV genomes were detected in all lymphoproliferative tumors. Immunophenotyping confirmed monoclonal gene rearrangements consistent with B-cell lymphoma, and global gene expression patterns correlated well with other human lymphomas. The ability of rituximab, an anti-CD20 antibody, to suppress human lymphoproliferation from a patient's ovarian tumor in SCID mice and prevent growth of an established lymphoma led to a practice change with a goal to reduce the incidence of lymphomas. A single dose of rituximab during the primary tumor heterotransplantation process reduced the incidence of CD45-positive cells in subsequent PDX lines from 86.3% ($n = 117$ without rituximab) to 5.6% ($n = 160$ with rituximab), and the lymphoma rate declined from 11.1% to 1.88%. Taken together, investigators utilizing PDX models for research should routinely monitor for lymphoproliferative tumors and consider implementing methods to suppress their growth.

Neoplasia (2017) 19, 1–9

Introduction

Ovarian cancer is the most lethal gynecologic malignancy with heterogeneous biology and unpredictable clinical behavior [1]. The development of animal models to recapitulate human disease is critical for understanding its complex biology and to develop novel therapies. Classic cell lines derived from tumor tissue not only lack *in vivo* peritumoral components but contain significant genomic differences from high-grade serous ovarian cancer [2]. In contrast, ovarian cancer patient-derived xenograft (PDX) models closely recapitulate the histologic, molecular, and drug response characteristics of the matched primary patient tumor [3]. Accordingly, interest in PDXs for preclinical drug development has led to multi-institutional

Abbreviations: PDX, patient-derived xenograft; TALs, tumor-associated lymphocytes.

Address all correspondence to: S. John Weroha MD, PhD, Division of Medical Oncology, Mayo Clinic College of Medicine, 200 First St. SW, Rochester, MN 55905
E-mail: Weroha.Saravut@mayo.edu

[1]Authors' disclosure of potential conflicts of interests: none.
[2]Support: This research was supported by the Mayo Clinic Specialized Program in Research Excellence grant CA136393 and R01 CA184502 from the National Institutes of Health, Ovarian Cancer Research Fund Alliance, Minnesota Ovarian Cancer Alliance, and a private donation from the Petersen Family to support general lab efforts.

[3]Present address: Medical Science Liaison, US Medical Affairs, Takeda Oncology, MN.
[4]Present address: Merck Research Laboratories, Merck & Company, NJ

Received 12 February 2017; Revised 20 April 2017; Accepted 24 April 2017

1476-5586
http://dx.doi.org/10.1016/j.neo.2017.04.007

and international efforts to develop PDX models to better understand cancer biology and develop better therapies [4,5].

A potential pitfall with creating and using PDX models is the potential for unintended co-heterotransplantation of non-carcinoma cell types. In ovarian cancer PDX models, tumor-associated plasma B cells can be detected by immunohistochemistry (IHC) of early-passage PDX tissue [6,7], and the presence of circulating human immunoglobulin (Ig) G can be detected for up to 4 months after implantation [6]. Although this might be desirable for investigations of immunotherapies in these models, the potential for neoplastic transformation of lymphocytes or spontaneous transformation of murine lymphocytes [8] can confound PDX-based *in vivo* studies. Indeed, examples of human lymphocytes transformed in immunocompromised murine hosts after heterotransplantation with adenocarcinoma has been reported for PDXs derived from lung, colon, gastric, liver, breast, bladder, renal, and prostate cancer [9–13]. Although preliminary data with similar findings have been previously presented in ovarian PDX models [14], to our knowledge, this process has not been well characterized in ovarian PDX tissues, and strategies to manage lymphocyte transformation have not been reported.

Herein we describe our experience with 277 ovarian tumor transplants in severe combined immunodeficiency (SCID) mice. Although the majority of PDXs recapitulate the histologic, molecular, and therapeutic response of the source patient tumor [3], a subset of PDX tumors is CD45 positive and represents a clonal outgrowth of malignant B cells which are inhibited by rituximab without impacting ovarian tumor engraftment.

Materials and Methods

Tumor Engraftment and Cryopreservation

Fresh tissues from consenting, chemotherapy-naïve patients with ovarian, primary peritoneal, or fallopian tube cancer were collected at the time of primary debulking surgery at Mayo Clinic, Rochester. All biospecimens were coded with a patient heterotransplant (PH) number to protect patient identity in accordance with the Mayo Clinic Institutional Review Board and in accordance with the Health Insurance Portability and Accountability Act regulations through the Mayo Clinic Ovarian Tumor Repository. Tumorgrafts were developed as previously described by intraperitoneal injection into female SCID beige mice (C.B.-17/IcrHsd-*Prkdcscid Lystbg*; ENVIGO) [3], also in accordance with the Mayo Clinic Institutional Animal Care and Use Committee. Briefly, ~0.3 ml of minced fresh patient tumor was mixed 1:1 with McCoy's media in a 1-ml syringe and injected intraperitoneally through a 0.5-inch 16-gauge needle. No enzymatic or mechanical tumor dissociation was performed. Mice were monitored by routine palpation for engraftment, and tumors were harvested when moribund.

Tissue Processing and IHC

Tissues collected from mice or patients were fixed overnight in buffered formalin (Cat#23-011-120; Thermo Scientific, Wilmington, DE) and processed in the tissue core facility at Mayo Clinic, AZ. Deparaffinized and rehydrated 5- to 6-μm sections were unmasked for 15 minutes in EDTA buffer (1 mM EDTA, 0.05% Tween 20, pH 8.0) at 95°C to 99°C. Primary antibodies to determine epithelial and lymphocytic populations were purchased from Life Technologies (pan-cytokeratin clone AE1/AE3 at 1:300) and Dako North America (CD45 clone 2B11 + PD7/26 at 1:500, human specific) and incubated overnight at 4°C. Secondary antibody (Cat#8125S; Signal Stain Boost IHC detection system; Cell Signaling Technologies, Schwalbach, Germany) was applied for 30 to 60 minutes at room temperature. Chromogenic detection of protein expression was determined in the presence of DAB (Cat#DS900H, Betazoid DAB Chromogen Kit; Biocare Medical, Concord, CA) and visualized by light microscopy. A DakoCytomation autostainer was used for anti-CD20 (Cat# N1502; Dako North America, Carpinteria, CA) and anti-CD3 (Cat# A0452; DakoCytomation). The slides were removed from autostainer, rinsed, and counterstained with hematoxylin.

RNA Hybridization and Microarray Clustering

Purification of genomic RNA and DNA was simultaneously extracted following the manufacturer's protocol for Qiagen AllPrep DNA/RNA mini Kit (Cat#80204; Qiagen, Venlo, Netherlands). Nucleic acid concentration and purity were determined on a Thermo Scientific NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, DE). All samples met RNA integrity number and validated Agilent (Agilent Technologies, Santa Clara, CA) criteria. Total tumorgraft RNA was analyzed by Affymetrix HG U133 plus 2.0 arrays at the Mayo Medical Genome Facility according to the manufacturer's protocol.

Gene expression arrays were preprocessed and normalized by robust multichip analysis [15]. For differential gene expression studies, epithelial versus lymphoma tumorgrafts were compared using the linear models and empirical Bayes methods that share information across genes to estimate variance (LIMMA) [16,17] and Java TreeView [18,19]. Study samples and the Stanford NCI60 Cancer arrays underwent unsupervised, hierarchical clustering. The NCI 60 contains expression data for 64 cancers, including ovarian and leukocyte derived (lymphoid). Tree-structured dendrograms and heat maps were surveyed in an unsupervised fashion for clustering.

Epstein-Barr Virus Detection

Nucleic acid from each tissue specimen was extracted into elution buffer using the MagNA Pure (Roche Diagnostics, Indianapolis, IN) Total Nucleic Acid program. Quantitation for the presence of the Epstein-Barr virus (EBV) DNA was accomplished with the LightCycler 2.0 EBV Quant Kit (Roche Molecular Diagnostics, Pleasanton, CA) using standard real-time polymerase chain reaction (PCR) cycling conditions.

Lymphocyte Clonality Studies

Clonality assessment was used to discriminate between malignant and reactive lymphoproliferation. Final molecular clonality was interpreted using clinical, morphologic, and immunophenotypic features in collaboration with hematologists, pathologists, and immunologists [20]. Using a B-cell immunophenotyping PCR assay, 39 fluorescently tagged primers amplified microsatellite loci (InVivoScribe Clonality Assay, San Diego, CA). The primers were designed to amplify all theoretical rearrangements of the Ig heavy and Ig kappa light chain genes. Amplicon product fragments were separated by capillary gel electrophoresis using the Applied Biosystems machinery. Microsatellite analysis reported mean fluorescence intensity of base pair size fragments using Gene Mapper Software (Life Technologies, Carlsbad, CA). Immunoglobulin gene rearrangements producing clonal peaks were classified as monoclonal/oligoclonal or polyclonal when present in established base pair size ranges.