The American Journal of Pathology, Vol. 186, No. 6, June 2016

The American Journal of
PATHOLOGY
ajp.amjpathol.org

GASTROINTESTINAL, HEPATOBILIARY, AND PANCREATIC PATHOLOGY

Isolation of Pancreatic Cancer Cells from a Patient-Derived Xenograft Model Allows for Practical Expansion and Preserved Heterogeneity in Culture

Kien Pham,* Daniel Delitto,† Andrea E. Knowlton,‡ Emily R. Hartlage,‡ Ricky Madhavan,* David H. Gonzalo,* Ryan M. Thomas,† Kevin E. Behrns,† Thomas J. George, Jr.,§ Steven J. Hughes,† Shannon M. Wallet,§ Chen Liu,* and Jose G. Trevino†

From the Departments of Pathology, Immunology, Laboratory Medicine, Surgery,† Oral Biology,‡ and Internal Medicine,§ Colleges of Medicine, Dentistry, and Public Health and Health Professions, University of Florida Health Science Center, Gainesville, Florida*

Accepted for publication February 16, 2016.

Address correspondence to Jose G. Trevino, M.D., Department of Surgery, College of Medicine, University of Florida Medical Center, Room R6116, Shands Hospital, 1600 SW Archer Rd., Gainesville, FL 32610; or Chen Liu, M.D., Ph.D., Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida Health Science Center, Room M651, Shands Hospital, 1600 SW Archer Rd, Gainesville, FL 32610. E-mail: jose.trevino@surgery.ufl.edu or liu@pathology.ufl.edu.

Commercially available, highly passaged pancreatic cancer (PC) cell lines are of limited translational value. Attempts to overcome this limitation have primarily consisted of cancer cell isolation and culture directly from human PC specimens. However, these techniques are associated with exceedingly low success rates. Here, we demonstrate a highly reproducible culture of primary PC cell lines (PPCLs) from patient-derived xenografts, which preserve, in part, the intratumoral heterogeneity known to exist in PC. PPCL expansion from patient-derived xenografts was successful in 100% of attempts (5 of 5). Phenotypic analysis was evaluated with flow cytometry, immunofluorescence microscopy, and short tandem repeat profiling. Importantly, tumorigenicity of PPCLs expanded from patient-derived xenografts was assessed by subcutaneous injection into nonobese diabeteic.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ mice. Morphologically, subcutaneous injection of all PPCLs into mice yielded tumors with similar characteristics to the parent xenograft. PPCLs uniformly expressed class I human leukocyte antigen, epithelial cell adhesion molecule, and cytokeratin-19. Heterogeneity within each PPCL persisted in culture for the frequency of cells expressing the cancer stem cell markers CD44, CD133, and c-Met and the immunologic markers human leukocyte antigen class II and programmed death ligand 1. This work therefore presents a reliable method for the rapid expansion of primary human PC cells and, thereby, provides a platform for translational investigation and, importantly, potential personalized therapeutic approaches. *(Am J Pathol 2016, 186: 1537–1546; http://dx.doi.org/10.1016/j.ajpath.2016.02.009)*

Pancreatic cancer (PC) is projected to be the second leading cause of cancer deaths by 2030.[1] Systemic cytotoxic and kinase-targeted regimens represent the standard of care for most patients presenting with PC. Most tumors, however, will develop rapid resistance to these regimens and will continue to progress by unknown mechanisms.[2,3] As a result, both the median survival and annual death rate for patients with PC have remained unchanged over the past 20 years.[4] To this end, an analysis of phase 1 cancer trials, which used agents with demonstrated efficacy in models derived from established commercially available cancer cell lines, revealed an overall objective response rate in only 3.8% of patients. For the past 40 years, commercially available PC cells (PCCs) such as MIA-PaCa2 (established in 1975),[5] CFPAC-1 (liver metastases established from a cystic fibrosis patient in 1990),[6] PANC-1 (established in 1975),[7] and BxPC-3 (*K-ras* wild-type established in 1986)[8] have been widely used in PC models and derived from an extremely small set of PC patients from >30 years ago. Thus, the poor predictive value of studies with the use of established cancer cell lines is a major barrier to the development of new interventions.[9,10] Our understanding of PC responses to therapy is also complicated by the marked molecular heterogeneity that exists among

Supported by National Cancer Institute grant 5T32CA106493-09 (D.D.) and Cracchiolo Foundation grant UF115477 (D.D.).

K.P. and D.D. contributed equally to this work.

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2016.02.009

primary tumors from different patients and intrapatient tumor cell heterogeneity, neither of which can be captured with currently available commercial cell lines.[11]

To begin to address some of these barriers, groups have attempted direct isolation and culture of patient-derived primary human PCCs from viable surgical specimens. The success rate of this method was reported to be approximately 10% in experienced hands,[12] although most groups do not publish failed attempts.[13–16] Conversely, in other cancer types, cell line derivation from patient-derived xenotransplantation demonstrates up to 10 times the success rate compared with that from cancer specimens at the time of resection.[17–20] Thus, to address these limitations, we leveraged our recently developed PC–patient-derived xenograft (PDX) model, whereby we have demonstrated that the early PDX is morphologically similar to the original cancer and retains both interpatient and intrapatient heterogeneity of the human disease.[17,21]

Specifically, here, we demonstrate the expansion of human PCCs through a PDX model that preserves the tumor heterogeneity with a 100% success rate. These established PDX-derived primary cell lines display uniform markers associated with a human PC origin with retained tumorigenicity. In addition, after several passages, these PCCs continue to exhibit significant heterogeneity in the expression of markers associated with their initial molecular phenotype and PC-immune cell interactions. In summary, this method of primary PCC isolation may provide a critical model that enhances clinical relevance by augmenting preclinical investigations and affording a personalized therapeutic examination.

Materials and Methods

Ethical Statement

Informed written consent was obtained from all patients, and the collection of all patient material was approved by the University of Florida Institutional Review Board. All animal studies were performed in accordance with the guidelines of the University of Florida Institutional Animal Care and Use Committee.

Histologic Analysis

Human PC specimens and all xenografts were evaluated by expert gastrointestinal pathologists, in accordance with the World Health Organization Classification of Tumors of the Digestive System. Tumors were classified according to site of origin and tumor stage.

Tumor Transplantation

The implantation of surgical tumor tissue into immunocompromised mice was described previously.[21] Briefly, a viable portion of resected tissue 2 × 2 mm in size was isolated immediately from surgically resected primary PC specimens with care to minimize critical ischemia time. PC tissue was then implanted subcutaneously into an 8-week-old female nonobese diabetic.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mouse (The Jackson Laboratory, Bar Harbor, ME). Xenografts were allowed to grow to a maximum diameter of 1.5 cm before passage and/or *in vitro* culture.

Isolation and Propagation of Patient-Derived PCC Populations

Tissue collected from xenograft tumors was minced into small pieces and enzymatically dissociated into single cells with 2 mg/mL STEMxyme 1 Collagenase/Neutral Protease solution (Worthington Biochemical Corporation, Lakewood, NJ) for 30 minutes at 37°C. After washing with Hank's balanced salt solution that contained 2% of fetal bovine serum, cells were filtered through a 100-μm filter to remove nondissociated cell clumps. The single cell suspension was then cultured in advanced Dulbecco's Modified Eagle Medium with nutrient mixture F12, 10% fetal bovine serum, 6 mmol/L glutamine, 1% of penicillin/streptomycin, and 40 ng/mL dexamethasone, at a density of 10^5 cells/mL on culture plates coated with rat tail collagen I. Media were changed every second or third day, and cells were passaged with Accutase (Innovative Cell Technologies, San Diego, CA) at 80% confluence. To avoid the outgrowth of contaminating fibroblasts, differential trypsinization was performed until a homogenous population of cytokeratin (CK)19/class I human leukocyte antigen (HLA)-expressing human PCCs was achieved by flow cytometry analysis.

Isolation of Nucleic Acid, DNA Fingerprinting, and Somatic Mutation Detection

DNA fingerprinting assays were performed to establish a unique genetic identification for each patient's PCC population. Total genomic DNA was extracted from cell pellets with Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's protocol, and sent to the Sanger Sequencing Core at the University of Florida for cell line authentication. The identity of the DNA profiles was determined by short tandem repeat profiling with the use of GenePrint 10 System (Promega, Madison, WI). This kit amplifies nine short tandem repeat human loci (*TPOX*, *vWA*, *D21S11*, *TH01*, *CSF1PO*, *D16S539*, *D7S820*, *D13S317*, and *D5S818*) and *AMEL* for sex identification, which was cross-referenced against patient sex. The samples were processed on an ABI 3130XL Genome Analyzer (Applied Biosystems, Foster City, CA), and data were analyzed with GeneMapper software version 4.0 (Applied Biosystems). Fingerprints were then compared with an established database of DNA profiles in the ATCC (Manassas, VA) to verify uniqueness.

Isolated DNA was also profiled for 38 somatic mutations within the following genes associated with PC: *APC*, *BRAF*,