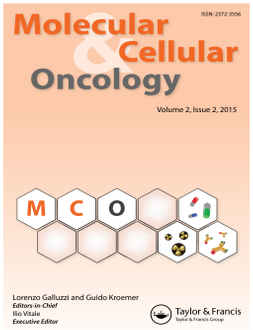

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Model organoids provide new research opportunities for ductal pancreatic cancer

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We recently established organoid models from normal and neoplastic murine and human pancreas tissues. These organoids exhibit ductal- and disease stage-specific characteristics and, after orthotopic transplantation, recapitulate the full spectrum of tumor progression. Pancreatic organoid technology provides a novel platform for the study of tumor biology and the discovery of potential biomarkers, therapeutics, and personalized medicine strategies.

Pancreatic ductal adenocarcinoma (PDA) is one of the most deadly malignancies. Current models for PDA consist of monolayer cell lines, patient-derived xenografts, and genetically engineered mouse models. Cell line experiments have yielded advances in our genetic understanding of this cancer, but do not recapitulate the physiologically relevant disease status in patients when orthotopically transplanted[1] and fail to predict therapeutic responses.[2] To overcome this deficiency, xenograft models and genetically engineered mouse models have been proposed as preclinical models for PDA.[3] However, these models are very expensive and time consuming to generate. Therefore, we set out to create a new model system that would facilitate progress in our ability to diagnose and treat patients with pancreatic cancer.

We previously developed methods to culture normal cells from mouse tissues such as small intestine,[4] liver,[5] and pancreas[6] as 3-dimensional organoids. We applied similar approaches to grow human tumor cells from colon[7] and prostate[8] as organoid cultures. Unlike monolayer cell lines, these cultures can be generated with high efficiency from untransformed cells and can be readily propagated under defined culture conditions. In this study, we have expanded on our previous studies to develop conditions that allow culture of both normal pancreatic duct cells and PDA cells from mice and humans as organoids.[9]

We have established murine ductal cultures from normal pancreas, pancreatic intraepithelial neoplasm (PanIN), PDA, and metastases. These cells proliferate in the presence of a defined set of growth factors and differentiation modulators that support the propagation of both normal and neoplastic cells within Matrigel, a 3D growth support matrix. Previously, it was not possible to culture premalignant stages of disease. Our ability to culture PanIN-derived cells will enable evaluation of these earlier stages of pancreatic cancer for putative biomarkers.

Orthotopic transplants of organoid cultures recapitulate the histology of the tissues from which they were derived. Wild-type organoids formed normal ductal structures following transplantation. In contrast, transplanted neoplastic PanIN or tumor organoids formed low-grade PanIN-like lesions and eventually progressed to invasive PDA. This organoid transplantation model will facilitate *in vivo* studies in situations where access to more traditional, genetically engineered mouse models of pancreatic cancer is not available.

We also performed transcriptomic and proteomic analyses of murine normal, PanIN, and PDA organoid cultures and identified changes specific to the premalignant and malignant disease states. Before this work, comparing tumor and normal tissue to identify such changes has been difficult because of the predominance of stromal cells in primary pancreatic tumors and the absence of an *in vitro* model system that allows the expansion of normal cells. We now report numerous genetic pathways that are altered during pancreatic cancer progression. Our data have identified many exciting candidate biomarkers, as well as pathways associated with pancreatic cancer development, that may have therapeutic relevance.

By modifying our techniques, we developed a strategy to also propagate human-derived normal and PDA organoids (**Fig. 1**). Human PDA organoids can be derived from either biopsies or surgically resected human pancreatic tumor specimens. The fact that human PDA organoids can be established from biopsies should be emphasized. Since only 15% of pancreatic cancer patients are eligible for surgical resection of their tumors,[10] the ability to establish organoids from biopsies will enable a broader sampling of the PDA patient population and iterative

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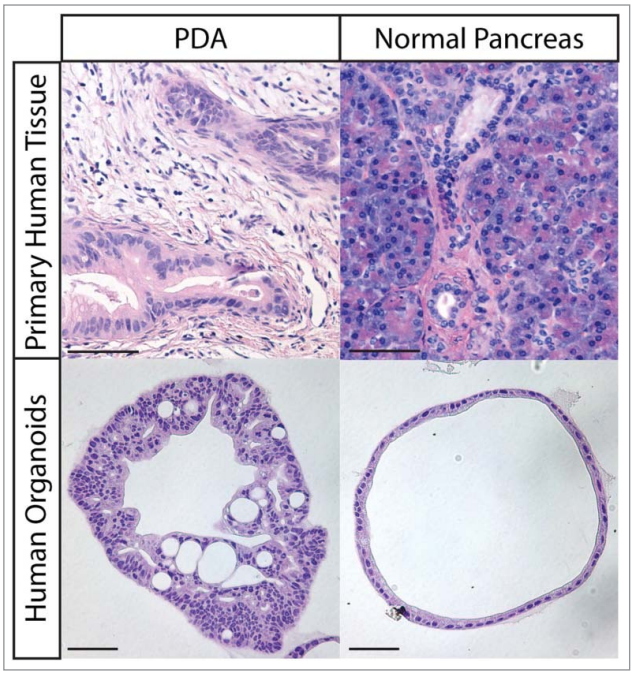

Figure 1. Organoids from healthy and neoplastic pancreas. Resected pancreatic tissues (top) and derived organoids (bottom). Hematoxylin and eosin staining; scale bars = 100 microns.

re-sampling of patients to longitudinally follow the course of their disease. Of note, the transplants developed in a distinct histological pattern, including stages that resemble early neoplastic disease. This differs from traditional transplantation models, which rapidly generate aggressive tumors without intermediate stages. Furthermore, other transplantation models do not recapitulate the hypovascular, stroma-rich microenvironment that often confounds the therapeutic response in pancreatic cancer patients. In contrast, our transplantation model accurately mimics this desmoplastic reaction and other physiologically relevant aspects of the human disease.

The high efficiency of generating organoid models within a timeframe of several weeks offers a significant advance over the isolation of 2D cell lines, which often fails, and the generation of patient-derived xenografts, which requires 6–12 months. In this regard, the organoid model will greatly facilitate efficient sampling of large patient populations that were previously inaccessible using traditional approaches. Therefore, this system allows the development of personalized approaches for pancreatic cancer treatment by providing a platform for identifying the best therapy for a given patient. These human organoids will now be tested to determine whether they can be used to identify therapeutic strategies for specific patients.

Taken together, the development of organoid culture methods for normal and neoplastic pancreatic tissues from mouse and human provides robust, stage-specific, cellular models and will be a valuable resource for the field. The ability to transplant genetically modified organoids provides a platform to evaluate molecular alterations responsible for the progression of low-grade lesions to invasive neoplasms. In addition, the organoid model system will allow investigation of potential diagnostic and therapeutic approaches.

Disclosure of Potential Conflicts of Interest

S.B. and H.C. are inventors on patents related to the subject.

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