Organoid cultures from benign and malignant prostate epithelium

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Background

Recent advances in genomics technology have highlighted the fact that cancer is an exceedingly heterogeneous disease, with each tumor harboring different sets of genetic aberrations. Biomarker development for the prediction of drug response relies in part upon accurate tumor models which can recapitulate the genomic diversity of the primary tumor, predict drug response in the patient, and be suitable towards high-throughput screening. Towards this end, cancer cell lines and patient-derived xenografts have been the primary biologic models for the mechanistic exploration of tumorigenesis and preclinical prediction of drug response. However, there are a number of limitations to both of these modeling systems.

Cancer cell lines possess obvious advantages towards cancer research, including indefinite growth and ability to perform high-throughput screening, although the efficiency to establish cell lines from patient-derived tumor tissue is highly dependent on the tumor type. In particular, prostate cancer has been notoriously difficult to establish representative cell lines. Therefore, particular subsets of a tumor that can grow in vitro are selected out. This selection process results in cell lines which do not adequately represent the tumor heterogeneity within individual patients and across the cancer type. Additionally, extensive passaging over time often results in loss of diversity as the cell line adapts to the culture conditions (1). Through genetic or epigenetic mechanisms, cancer cell lines lose differentiation characteristics and gain corresponding increased proliferative abilities, often within several passages (2).

To overcome some of the limitations of cancer cell lines, patient-derived xenografts (PDX) have been utilized as a more physiologic model of patient tumors. Studies suggest that PDX models maintain tumor heterogeneity across passages, allowing for therapeutic precision medicine strategies like drug response prediction (3–5). Unfortunately, these models are limited by the need for large quantities of tumor tissue—often obtainable only by surgical means—as well as high engraftment failure rates (6, 7). Additionally, PDX models are not amenable to high-throughput screening or genetic manipulation studies and often take over six months for engraftment and drug treatment response results, which limits the clinical utility of these models. Human prostate cancer is also very inefficient in the ability to establish PDX models that can grow and be serially passaged.

Recently, three-dimensional cancer "organoid" culture methods have been developed to establish patient-derived cancer lines at increased efficiency and without some of the limitations.
of PDX and cancer cell line models. Due to improvement in genomics technologies, the newly established models are well-annotated clinically and genetically, therefore improving their utility in biomarker development. Organoid culturing technology offers the promise to establish a large repertoire of \textit{in vitro} models that recapitulate disease heterogeneity for a number of different cancer types for large scale drug screening and fundamental cancer research. The development of organoid culturing technology may provide a promising strategy for precision medicine in the future.

\textbf{Discussion}

Organoid culturing conditions were first developed for benign intestinal epithelial cells, where single stem cells cultured in the appropriate serum-free conditions grew into three-dimensional epithelial structures with the correct crypt-villus structure of normal intestinal epithelium \cite{8}. Since these three-dimensional epithelial structures encompassed all of the component cell types of normal intestinal epithelium and accurately recapitulated intestinal histology and differentiation, they were called “organoids.” Organoids were further defined as self-organized structures which contain several cell types derived from stem cells or organ progenitors and which, similarly to the process which occurs \textit{in vivo}, undergo cell sorting and spatially-restricted lineage commitment \cite{9}. Organoids have since been developed to model colon, stomach, liver, thyroid, pancreas, and prostate tissue among others \cite{10–15}.

Organoids are able to propagate indefinitely without senescence and, like cancer cell lines, are able to be genetically manipulated. For instance, intestinal organoids derived from patients with cystic fibrosis have recently undergone genome editing with the CRISPR/Cas9 system resulting in repair of the mutant \textit{CFTR} gene and restoration of normal secretory function \cite{16}. To model tumorigenesis in the human setting, benign intestinal organoids were engineered with the \textit{KRAS}^{G12V} mutation and with deletion of \textit{APC}, \textit{SMAD4} and \textit{P53} \cite{17}. These engineered tumor organoids recapitulated human colorectal cancer. Organoid culturing has also provided insights toward lineage plasticity and the identification of epithelial stem cell compartments \cite{15}.

The success of organoid culturing in multiple benign epithelial types has led to great interest in the creation of patient-derived organoid cancer models. Multiple published and unpublished efforts are underway to create well-annotated organoid cancer lines in multiple cancer types, including prostate, pancreas, colorectal, and lung \cite{14, 18}. Of particular interest is prostate cancer which, despite being the most common solid tumor in men in the United States, had only seven publically-available cell lines prior to the development of organoid culturing. Furthermore, these cell lines did not recapitulate the common genomic events found in human prostate cancers, including \textit{TMPRSS2-ERG} interstitial deletion and \textit{SPOP} mutations. The recent establishment of seven novel organoid prostate cancer lines not only doubled the previously existing number of cancer lines in a short period of time, but also included detailed clinical and genomic annotation, including treatment history and disease course, whole exome sequencing to identify somatic mutations, array comparative genomic hybridization to determine copy number aberrations, and paired-end RNA sequencing to detect fusion events and characterize the transcriptional landscape \cite{18}. These novel organoid prostate cancer lines were developed from a number of tissue sources including circulating tumor cells, were genomically diverse, and also captured a number of prostate cancer-specific genomic events.
Additionally, they were found to be amenable to drug testing, engraftment, and genetic manipulation. While experience with organoid technology in the setting of cancer is limited, there is great potential to better model, study, and treat human malignancies.

Future Directions

The continued push towards precision medicine in clinical oncology combined with improvements in culturing technology has provided the opportunity to greatly expand the number of cancer models with much improved recapitulation of the heterogeneity of human cancer. Continued optimization of culturing conditions to increase the efficiency of organoid line establishment is essential. Exploratory drug response data in prostate cancer organoid lines seem to match with actual patient clinical responses, but it will be important to continue to determine the fidelity of in vitro predictions to actual patient drug response data (18). Due to the rich concentration of growth factors like EGF in organoid media, it is possible that certain targeted therapies (i.e. EGFR inhibitors) may not be accurately represented by in vitro drug response data. Furthermore, it is also possible that cancers may lose dependence upon driver oncogenes in the setting of continued external growth stimulus from the culture conditions. Certainly, a large number of cancer models for each tumor type will be necessary to accurately represent the diversity of each cancer, as well as to validate drug response data, assist in biomarker discovery, and to power the development of novel, precision therapies.

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Organoids

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