Characterization of Patient-Derived Pancreatic Ductal Adenocarcinoma Organoids Through Detection of Oncogene *KRAS* Mutations

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1. Abstract

In collaboration with Hirslanden Clinic patient-derived organoids of pancreatic ductal adenocarcinoma, a highly aggressive form of cancer, were cultured as part of a feasibility study. DNA was extracted and amplified for *KRAS*^{G12} and *KRAS*^{Q61} and Sanger sequenced, alongside the parental tissue. While some samples yielded congruent results, some were more unclear, which was attributed to low variant allele frequency. Due to the heterogeneity of cell population, possible mutations were below detection threshold. Genetic diversity complicates Sanger detection but enhances organoids' value as preclinical models by better mimicking in vivo conditions.

Furthermore, organoids were cultured in growth factor depleted media to study a possible enrichment of KRAS mutant cells.

2. Introduction

The Translational Organoid Resource Core (TOR) develops, stores, and optimizes patient-derived organoids, currently focusing on bladder cancer and pancreatic adenocarcinoma. Organoids are 3D structures replicating key structural and functional features of organs. (cf. Fig.1) In partnership with Hirslanden Clinic in Zurich, TOR is conducting a feasibility study to assess pancreatic adenocarcinoma organoids as in vitro models for predicting therapy response.

Pancreatic carcinoma is a highly aggressive solid tumor with poor prognosis, late detection, and limited treatment options. Its most common form, pancreatic ductal adenocarcinoma (PDAC), arises in the exocrine pancreas via multistep progression of premalignant lesions driven by genomic alterations. [1] The earliest known mutation is an activating *KRAS* mutation at codon 12, producing a constitutively active protein that triggers proliferative and survival pathways like MAPK. [2]

This thesis presents a preliminary analysis of *KRAS*^{G12} mutations in parental tissue (PT) and derived organoids (PDO). Results will be integrated with RNA and whole exome sequencing data from the Kruithof-de Julio group. Protein-level characterization of PT and PDO was planned but delayed due to late antibody delivery.

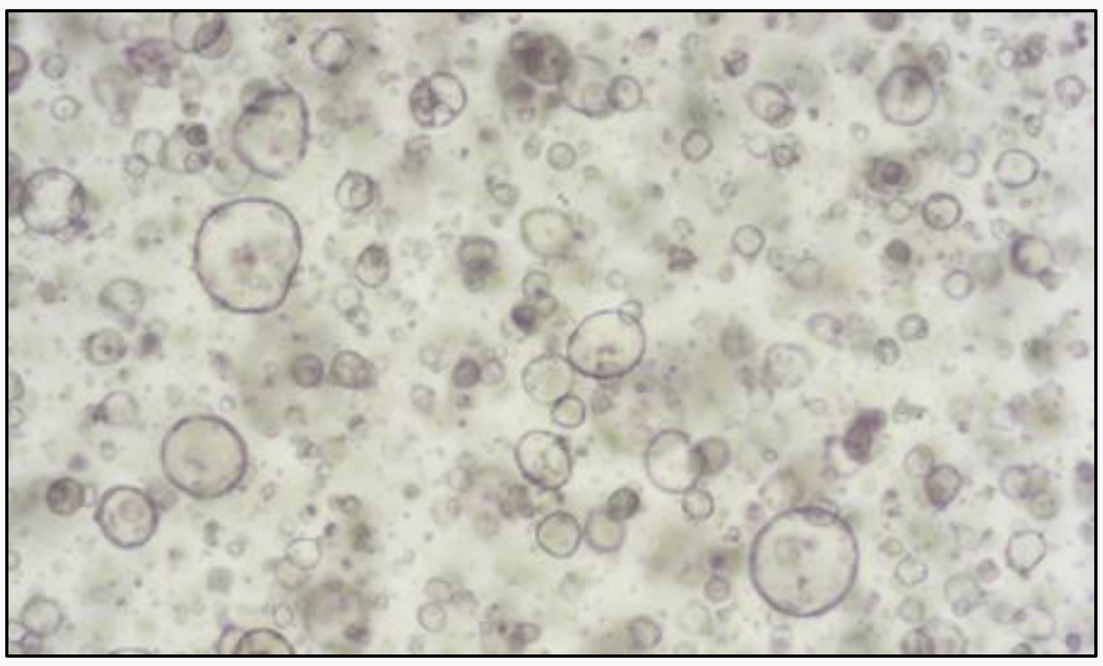

Figure 1 Representative image of PDAC organoids.

References

[1] Halbrook, C. J., Lyssiotis, C. A., Pasca Di Magliano, M., & Maitra, A. (2023). Pancreatic cancer: Advances and challenges. Cell, 186(8), 1729–1754. https://doi.org/10.1016/j.cell.2023.02.014

[2] Ardalan, B., Ciner, A., Baca, Y., Hinton, A., Darabi, S., Kasi, A., Lou, E., Azqueta, J. I., Xiu, J., Datta, J., Shields, A. F., Aguirre, A., Singh, H., Shroff, R. T., Pishvaian, M. J., & Goel, S. (2025). Distinct Mo-lecular and Clinical Features of Specific Variants of KRAS Codon 12 in Pancreatic Adenocarcinoma. Clinical Cancer Research, 31(6), 1082–1090. https://doi.org/10.1158/1078-0432.CCR-24-3149

Figure 1 Kramer, P. (2025). Representative image of PDAC organoids.

3. Aims and Leading Questions

Aim 1: Characterization of pancreatic ductal adenocarcinoma PDOs *KRAS* oncogene mutational landscape.

Leading Question 1: What is the mutational landscape of *KRAS* oncogene in PDAC PDOs available at the TOR?

Aim 2: Histological characterization of a subset of PDAC PDOs. The expression of key PDAC markers such as CK17, CK19, MUC1 and SP100 and proliferation marker such as Ki67 will be assessed by IHC and/or IF. When available the expression of these markers will be compared with the parental tissue to assess to what extent the PDOs resemble the parental tissue.

Leading Question 2: How are these models characterized at a protein level and to what extent they resemble the original tissue?

4. Methods and Materials

DNA was extracted using column-based methods (DNeasy®, Qiagen), and *KRAS* regions (codons 12 and 61) were amplified via PCR. Products were visualized by agarose gel electrophoresis and quantified using ImageJ, referencing a known DNA ladder band. Samples were sent to Microsynth for Sanger sequencing. Clear chromatograms showing either wild-type or a single mutation were compared to clinical data from Hirslanden Clinic, the occurrence of multiple peaks was labeled ambiguous.

Organoids were cultured from cryopreserved cells or from tissue digestion. About 4e5 cells were seeded in 200 µL Matrigel, forming ten domes per well. Well numbers varied by cell availability and experiment. Culture duration ranged from days to weeks. Viable lines were maintained as needed and excess was cryopreserved. Media was changed every two days.

EGF depletion effects on organoid growth were tested in select samples. DNA from these conditions was also sequenced via Sanger.

5. Results

The *KRAS*^{G12} status was reliably determined in eleven samples. Four samples yielded congruent results in PT and PDO. Three samples of clinically unknown status were found to be wild-type. The clinical status could be validated on four PDO samples.

The remaining eleven samples could not be characterized due to missing PDO DNA in four samples, five samples where the PDO status was different to the clinical status and one sample where results in PT and PDO were ambiguous.

All PDO samples were tested wild-type for *KRAS*^{Q61}.

Exploring the dependency on EGF it was found that in cases where it was possible to grow organoids in both conditions they grew better in medium containing EGF. Sequencing revealed two samples carrying a *KRAS* mutation.

6. Discussion

Inconsistent sequencing results may stem from cell population heterogeneity. Ambiguous chromatograms often reflect low variant allele frequency: in Sanger sequencing, mutations can be masked if most cells carry the wild-type allele. Detection typically requires ≥15–20% mutant alleles. For higher sensitivity, next-generation sequencing or digital PCR is preferred, though more costly.

Organoids with *KRAS*^{G12} wild-type regress under EGF depletion due to inactive MAPK signaling and halted growth. Genomic profiling may reveal malignant potential.

Higher passage numbers and *KRAS* mutant-rich tissue yielded more robust sequencing results. Genetic diversity complicates Sanger detection but underlines the value of organoids as preclinical models by better mimicking in vivo conditions.