Appendix 1

Methods

Organoid generation, establishment, and expansion

Previously established methodology was followed to establish and maintain PDOs (11). Briefly, patient tissue was minced and incubated in digestion media (1 mg/mL Collagenase XI, 10 µg/mL DNase I, 10.5 µmol/L Y-27632 in Human Complete Medium) at 37 ℃ with mild agitation for up to 45 minutes for resection specimens. Cells were washed in human organoid wash media (Advanced DMEM/F12, HEPES 10 mmol/L, Glutamax 1X, Primocin 1X, with 0.1% Bovine Serum Albumin). Cells were then plated in Matrigel domes and cultured in Human Complete Defined Media (Advanced DMEM/F12, HEPES 10 mmol/L, Glutamax 1X, A83-01 500 nmol/L, hEGF 50 ng/mL, mNoggin 100 ng/mL, hFGF10 100 ng/mL, hGastrin I 0.01X, *N*-acetylcysteine 1.25 mmol/L, nicotinamide 10 mmol/L, B27 supplement 1X, R-spondin1 10 nM, and NGS/Wnt 0.2nM) in an incubator at 37 ℃ and 5% CO_2.

Confluent PDOs were passaged according to standard protocols. Media was aspirated from the wells and Matrigel domes were broken down in Matrigel Melting Solution. Once completed, PDOs were dissociated to single cells using TrypLE™ (Gibco) for 5–15 minutes at 37 ℃, washed then plated on a new plate in fresh Matrigel domes and Human Complete Defined Media and returned to the incubator. Fresh media was added every 3–4 days.

DNA Extraction

PDOs were isolated from the Matrigel, washed and stored in dPBS with 0.1% BSA at –80 ℃ until the extraction was conducted. DNA purification was performed using the QIAamp Mini Prep Kit (Qiagen) protocol. In summary, PDOs were lysed with Proteinase K in a lysis buffer before the DNA was precipitated, washed, and eluted in Ultrapure Distilled Water. DNA quality and quantity were assessed using the Nanodrop 2000c and Qubit Fluorometer, respectively.

Pharmacotyping

Drug screening was performed following previously published methodology (11). Briefly, PDOs were passaged following standard protocol before single cells were passed through a 40 µm cell strainer. Cells were quantified using acridine orange and propidium iodide (AO/PI) staining (Nexcellom) and plated on pre-treated low-attachment plates. Plates were pre-treated with drugs in dose response curves using an ECHO 650 series acoustic liquid handler. Cell viability was assessed after 5 days of growth using CellTiter-Glo® Luminescent Cell Viability Assay (Promega) and fluorescence was read using an Envision plate reader. The data was normalized using controls (dimethyl sulfoxide (DMSO) & Staurosporine), analyzed, and non-linear fit dose response curves were produced using GraphPad Prism v 8.4.3. The area under the curves (AUC) was used to rank the sensitivity of the PDO compared to a previously published AUC biobank (11).

High-Content Imaging

Drug treatment was performed following previously published methodology (11). Briefly, PDOs were passaged following standard protocol before single cells were passed through a 40 µm cell strainer. Cells were quantified using AO/PI staining (Nexcellom). Half a million cells in Human Complete Defined Media with 5% Matrigel were plated in a 384-well plate. The organoids were grown for 7–11 days with a media top up after 5 days. Gemcitabine, paclitaxel, 5-fluorouracil, oxaliplatin, SN-38, RMC-6236 and afatinib were added onto the plates using an Echo 650 series acoustic liquid handler. PDOs were stained with AO/PI dye and imaged at 10X after a 5-day incubation period using the Molecular Devices Image Xpress Confocal HT.ai High-Content Imaging (HCI) System.

Tumor Validation and PDO sequencing

DNA samples from the PDO were sent overnight to the David M. Rubenstein Center for Pancreatic Cancer Research for MSK-IMPACT sequencing following previously published methodology (44). This sequencing platform analyzes 505 oncogenes, tumor suppressor genes, and pathway-associated genes that are actionable by current targeted therapies. Probes were designed using the NimbleGen SeqCap system, and captured libraries were sequenced on the Illumina NovaSeq 6000 platform.

https://dx.doi.org/10.21037/jgo-2025-aw-818

Data was processed using a customized analysis pipeline developed by Memorial Sloan Kettering Cancer Center to identify missense mutations, copy number alterations and structural variants.

Histology

To fix the PDOs, media was aspirated from the plate, and 10% neutral buffered formalin was carefully added to the Matrigel domes and incubated at 37 ℃ on a hot plate for 30 min. The formalin was then removed, and the domes were washed with dPBS. The plate was left to dry on the hot plate at 37 ℃ for 10 mins. The domes were lifted from the plate using a cell lifter and transferred to a tissue embedding cassette using a wide-orifice pipette tip. The cassette was placed in a histology jar containing 70% ethanol and sent to the Cold Spring Harbor Laboratory (CSHL) Histology Core for standard formalin-fixed paraffin embedded (FFPE) processing, and hematoxylin and eosin (H&E) staining. The images were scanned using the Olympus VS200 Slide Scanner.

References

44. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn 2015;17:251-64.

https://dx.doi.org/10.21037/jgo-2025-aw-818