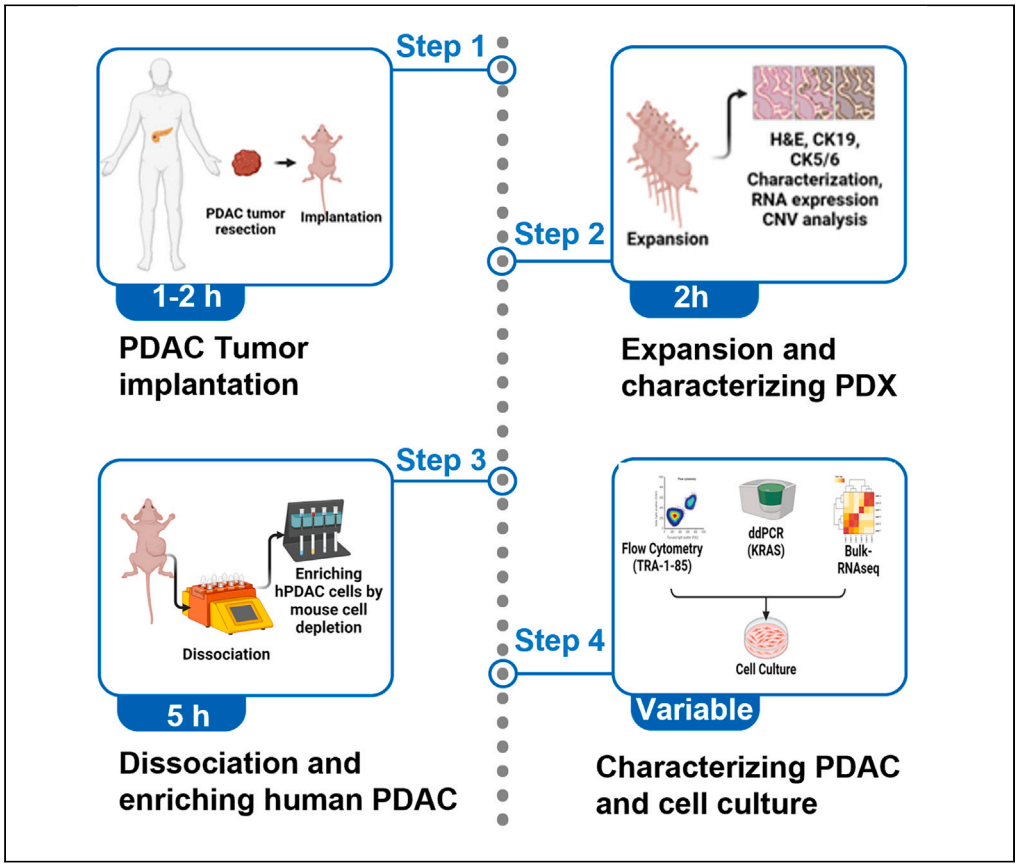
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol to purify and culture human pancreatic cancer cells from patient-derived xenografts



Pancreatic ductal adenocarcinoma (PDAC) exhibits extensive inter- and intratumoral heterogeneity, along with a significant stromal component. This protocol outlines steps to generate patient-derived xenografts (PDXs), isolate and enrich human PDAC epithelial cells, and verify their identity using droplet digital PCR (ddPCR) and human-specific markers. It provides a robust approach for culturing cancer epithelial cells to develop a human PDAC model system.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Julien Tessier, Dmytro Grygoryev, Marilynn Chow-Castro, ..., Terry Morgan, Rosalie C. Sears, Jungsun Kim

kimjungs@ohsu.edu

Highlights

Steps to expand patient-derived xenograft (PDX) tumors in mice

Details on extracting and dissociating tumors from patient-derived xenografts

Instructions for separating human pancreatic cancer cells from mouse fibroblast cells

Steps to characterize human pancreatic cancer cells derived from PDX

Tessier et al., STAR Protocols 6, 103672
March 21, 2025
https://doi.org/10.1016/j.xpro.2025.103672

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol to purify and culture human pancreatic cancer cells from patient-derived xenografts

Julien Tessier,[1,9,10] Dmytro Grygoryev,[1,2,9,10] Marilynn Chow-Castro,[1,9,10] Taelor Ekstrom,[1] Elise Manalo,[1] Seung-Won Lee,[1,2] Mark Berry,[1] Jason M. Link,[2,3] Dove Keith,[3] Brittany L. Allen-Petersen,[2,3] Brett Sheppard,[3,6] Terry Morgan,[1,7,8] Rosalie C. Sears,[2,3,8] and Jungsun Kim[1,2,8,11,*]

[1]Cancer Early Detection Advanced Research Center at Knight Cancer Institute, Oregon Health & Science University (OHSU) School of Medicine, Portland, OR 97201, USA

[2]Department of Molecular and Medical Genetics, OHSU School of Medicine, Portland, OR 97201, USA

[3]Brenden-Colson Center for Pancreatic Care, OHSU School of Medicine, Portland, OR 97201, USA

[6]Department of Surgery, OHSU School of Medicine, Portland, OR 97201, USA

[7]Department of Pathology, OHSU School of Medicine, Portland, OR, USA

[8]Cancer Biology Research Program, Knight Cancer Institute, OHSU School of Medicine, Portland, OR 97201, USA

[9]These authors contributed equally

[10]Technical contact

[11]Lead contact

*Correspondence: kimjungs@ohsu.edu
https://doi.org/10.1016/j.xpro.2025.103672

SUMMARY

Pancreatic ductal adenocarcinoma (PDAC) exhibits extensive inter- and intratumoral heterogeneity, along with a significant stromal component. This protocol outlines steps to generate patient-derived xenografts (PDXs), isolate and enrich human PDAC epithelial cells, and verify their identity using droplet digital PCR (ddPCR) and human-specific markers. It provides a robust approach for culturing cancer epithelial cells to develop a human PDAC model system.

For complete details on the use and execution of this protocol, please refer to Grygoryev et al.[1]

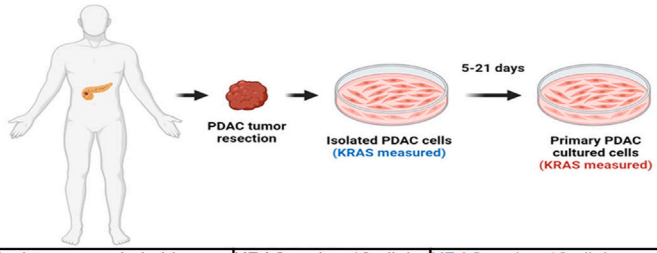
BEFORE YOU BEGIN

Pancreatic ductal adenocarcinoma (PDAC) exhibits extensive inter- and intratumoral heterogeneity, along with a significant stromal component.[2] Our previous research demonstrated that lentiviral transduction preferentially favors certain cell types, particularly normal cells, in human primary PDAC cultures.[1] Therefore, accurately assessing whether cultured cells reflect patient-derived cancer cells is essential. By analyzing tumor cells from individual patients, we discovered that only a small fraction of cells within a given PDAC tumor exhibit KRAS mutant alleles, even though more than 90% of PDAC patients harbor oncogenic KRAS mutations[3] (Figure 1). To overcome this limitation, we developed a method that enriches KRAS mutant PDAC cells using patient-derived xenografts (PDX) combined with mouse cell depletion. The following protocol details the steps for isolating and culturing KRAS mutant cells from patient-derived primary PDAC cells, leveraging the PDX model and enrichment strategies (Graphical Abstract).

Institutional permissions

All animal procedures for PDX tumors complied with the OHSU Institutional Animal Care and Use Committee (IACUC) under protocol IP00004592. Human PDAC specimens were obtained through

A



Patient #	pathological tumor grade in biopsy acquired	KRAS codon 12 allele frequencies by pyrosequencing from paraffin slide	KRAS codon 12 allele frequencies by pyrosequencing from isolated cells	KRAS codon 12 allele frequencies by pyrosequencing from primary cultured cells
#1	normal	ND	ND	wild (G)
#2	Well-differentiated	27%	ND	wild (G), G12D 2.7%, G12A 6%
#3	well differentiated tumor (25% enriched cancer)	ND	wild (G)90%, G12D 7%	wild (G) 92%, G12D 5%, G12A 3%
#4	poorly differentiated,	ND	wild (G) 94.1%, G12D 3.4%	wild GGT 82.3%, G12D 17.6%
#5	moderately differentiated	ND	wild G 60%, G12R 18%, G12A 15%. G12S 6%, G12D 3%	wild G 91%, G12S 6%, G12A 2.7%, G12D 1.5%
#6	Liver met-moderately differentiated	ND	ND	wild (G) 61%, G12D 39%
#7	moderately differentiated	ND	wild (G) 85.1%, G12D 8.4%, G12V 4.3%	wild(G) 83.5% , G12D 16.4%
#8	poorly differentiated	ND	wild (G) 91% , G12D 6.8%	wild(G) 83%, G12D 17%
#9	ND	ND	ND	ND
#10	A mixture of poorly differentiated tumors with well-differentiated tumors.	ND	wild (G)94%, G12D 2%, G12A 4%	ND

B

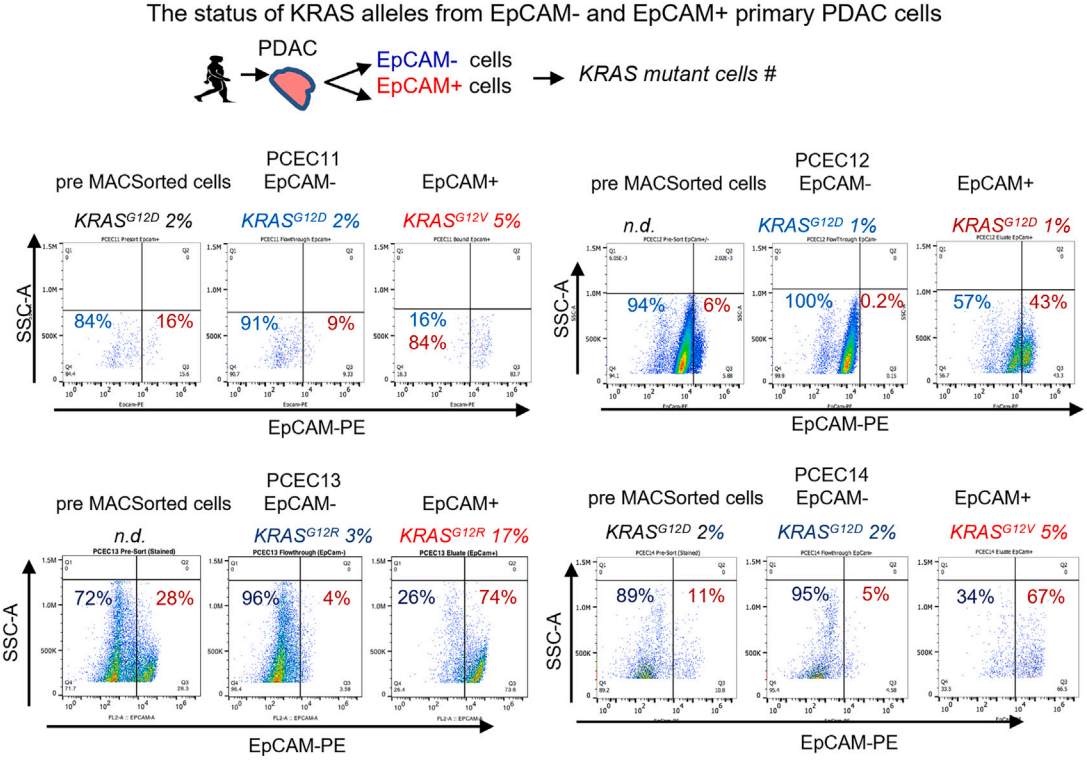


Figure 1. KRAS mutation frequency in patient-derived primary PDAC tissues
(A) Frequency of KRAS mutant alleles in primary PDAC cells immediately after tissue dissociation and initial culturing.
(B) Frequency of KRAS mutant alleles in EpCAM- and EpCAM+ primary PDAC cells. In both cases, a notable paucity of KRAS mutant cells is observed in primary human PDAC, suggesting a lower-than-expected frequency of KRAS mutations in primary PDAC tissues.

the Oregon Pancreas Tissue Registry study (IRB00003609), with all experimental protocols approved by the OHSU Institutional Review Board. Informed consent was obtained from all participants.

Implantation of human PDAC into NOD-SCID IL2Rgamma^{null} mice

Timing: 1–2 h

We bank PDAC tissues derived from patients by implanting them into NSG mice as previously described.[4] Obtain informed consent from all study participants and de-identify PDAC samples. The texture of highly fibrotic pancreatitis is similar to that of PDAC. Therefore, before transferring the tissue sample to the lab, a well-trained pathologist performs a quick H&E staining to verify the presence of tumors in the resected tissues and the tumor grade and the ratio of cancer-associated fibroblasts (CAFs) to epithelial cells. The ratio of CAF to epithelial cells is an important factor in primary culture, as CAF cells often dominate despite the presence of fibroblast growth inhibitors in our media. However, based on our experience, this ratio has minimal impact on the quality of PDX tumors, though it can delay tumor growth. Consequently, the tumor growth duration varies significantly depending on the parental tumors used for the initial xenograft. However, as the tumors are passaged in mice, this variability decreases. If deriving CAF cells is required, harvest them directly from primary PDAC tumors obtained from patients before implantation into mice, following the procedure outlined in Steps 2–4.

1. Aliquot Matrigel Matrix in 1 mL Eppendorf tubes.

   Note: Matrigel will start to gel above 10°C. Thus, always use pre-cooled pipets, tips, and tubes when handling Matrigel.

   ⚠ CRITICAL: To limit freezing-thaw cycles, make a single-use aliquot when the vial is initially thawed and then store it in a non-frost-free freezer at −20°C or lower. To minimize temperature fluctuation, do not store the aliquot in the freezer door. Do not freeze-thaw Matrigel more than five times.

   a. Thaw Matrigel at 4°C for 12–16 h.
   b. Pre-chill a box of tips and 1.5 mL tubes at −20°C.
   c. Bring ice into the tissue hood and place Matrigel on ice.
   d. Aliquot Matrigel into a single-use Eppendorf on ice (350–500 μL).
   e. Store it in a non-frost freezer at −20°C or lower.
2. Transport surgically resected tumor pieces for archiving by either injecting them into mice or freezing them in liquid nitrogen (LN2).
   a. Transport a piece of PDAC tissue in transporter media on ice from the surgical pathology lab or operating room to the laboratory.

   ⚠ CRITICAL: To prevent contamination during transport, place tissues in transport media containing antibiotics (see the "transport media" recipe in the materials and equipment setup). However, residual antibiotics have been observed to reduce tumor survival. Therefore, thoroughly remove the antibiotics by rinsing the tissues multiple times (at least three times) before proceeding with further processing (see step 4a).

   b. Archive a small piece for long-term storage in LN2.

i. Place a small piece in freezing media (10% DMSO + 90% FBS).
ii. Store it at −80°C for 12–16 h.
iii. Transfer the tumor piece to an LN2 tank for long-term storage.

Note: Surgically resected tumors can be stored for long-term use and future transplantation. While we did not have much success recovering cells directly from frozen tumors, we are able to recover tumors from frozen samples through PDX with nearly a 100% success rate.

c. Immediately transplant a piece of PDAC tumor into an NSG mouse (go to step 3).

3. Anesthetize the NSG mice using isoflurane.
   a. Set up mouse cages (1 per mouse) and label them accordingly.
   b. Set up the anesthesia chamber and cone with 2.5% isoflurane and prepare a heating pad at 37.5°C, as general anesthesia affects several body systems, including thermoregulation.

⚠ CRITICAL: The heating pad temperature should be kept at a safe level, and gel should be applied to the mouse's eyes to prevent them from drying out while on the heating pad.

   c. Place the mouse in the anesthesia chamber until anesthetized, then transfer to the cone.
   d. Check that the mouse is fully anesthetized by pinching the foot.

⚠ CRITICAL: To confirm unconsciousness and absence of pain perception, perform a paw withdrawal test by firmly pinching the paw with atraumatic forceps or fingers. Regularly monitor the mouse throughout the anesthetic and surgical procedure. Ensure proper breathing by continuously checking respiratory patterns during surgery and adjusting isoflurane levels as necessary.

4. Transplant tumor pieces into NSG mice (See problem 1).
   a. Tape ears on the cone. Shave the hair on the back or flank of the animal where the tumor will be transplanted.

⚠ CRITICAL: Ensure the mouse's ears are securely taped to the cone in a position that is out of reach of its feet. This prevents the mouse from scratching the sutures, which could compromise the implantation site.

   b. Sterilize the area using 70% ethanol, then betadine.
   c. Make a small incision in the skin (no more than 5 mm) and create a pocket using strabismus scissors (introduce -> open -> pull out -> close)
   c. Pick out the piece of tumor with forceps.
   d. Rinse it in DMEM with 10% fetal bovine serum (FBS) three times (See Critical above).
   e. Dip it in an aliquot of matrigel matrix kept on ice.
   f. Insert the tumor into the pocket as far as possible from the opening incision site.

⚠ CRITICAL: To maximize transplantation efficiency, limit the size of each tumor piece to 2–3 mm^3 per mouse. There is no minimum tumor size for injection; however, smaller tumor pieces may take longer to grow (see problem 1 for details).

   g. Close the incision with simple interrupted sutures.
5. Place the mouse back on the heating pad and wait for it to wake up.
6. Once the mouse wakes up, place it in a brand cage.

Note: House a maximum of 5 mice per cage and avoid mixing mice from different boxes. If signs of fighting or wounds are observed, isolate the affected mice into separate cages.

Monitor recovery by measuring each mouse's weight every two days for at least one week following surgery.

7. Check its recovery by measuring its weight every two days for at least a week after surgery.
8. Check tumor progression by measuring it with a caliper.

Expansion of PDX tumor from NSG mice

Timing: 2 h

We expand PDX tumors from NSG mice. To minimize potential genetic instabilities in mice, limit the *in vivo* expansion of PDX tumors to no more than five passages.[5] Rapid accumulation of copy number alterations (CNAs) during PDX passaging has been reported.[5] However, these changes are often the result of the selection of pre-existing minor clones in the original tumor, and the degree of genomic instability in PDX models mirrors that of the primary tumors.[5] Despite retaining many genetic features of the original tumors, PDX models have also been shown to develop divergent genomes over time.[5] Therefore, careful monitoring and validation are critical to ensure consistency with the original tumor characteristics. Genomic stability can be assessed through CNV analysis or karyotyping. To evaluate the similarity between PDX tumors and the original tumors, we compare histology using H&E staining, along with tumor subtype markers such as CK19 and CK5/6. Additionally, ddPCR and RNA sequencing of PDX tumors can be used to assess their molecular profiles against those of the corresponding parental PDAC cells (Figure 2).

⚠ CRITICAL: Avoid letting tumors grow beyond 1 cm^3 to maintain both the quality of life for the mice and the integrity of the tumors. Once a tumor reaches approximately 1 cm^3, extract it, cut it into small pieces, and expand it by transplanting it into 5 new NSG mice. Freeze the remaining tumor pieces for future use (refer to step 4).

9. Prepare the following reagents.
   a. Get one aliquot of Matrigel from −20°C and keep it on ice.
   b. Prepare freezing buffer (90% FBS + 10% DMSO).
   c. Freshly prepare 4% Paraformaldehyde (PFA In PBS).
   d. Prepare 1× Hank's Balanced Buffered Saline Solution (HBSS) with 25 mM HEPES and keep on ice.
10. Recover the tumor from the NSG mouse, keep it on ice, and process it as fast as possible much as possible.
    a. Anesthetize the mouse (isoflurane 2.5%) and shave the back.
    b. Open the skin with sterile dissecting tools and collect the tumor without damaging it.

⚠ CRITICAL: Minimize host tissue by carefully removing as much of it as possible.

c. Transfer tumors into a glass vial containing 25 mM HEPES/HBSS buffer on ice.

Note: Remove skin, blood and/or any necrotic area prior to cutting the tumor into tiny pieces (approximately 3 mm^3 each).

d. Rinse the tumor pieces with fresh, ice-chilled 25 mM HEPES/HBSS buffer on ice, and then continue with the following steps.
   i. Place one piece of tumor in ice-cold 4% PFA/PBS for histology.
   ii. After incubating at 4°C for 12–16 h, rinse 3 times in ice-cold PBS.
   iii. keep it at 4°C until embedding into paraffin.
   iv. Place up to 6 tumor pieces into the freezing buffer (10% DMSO/90% FBS).
   v. Transfer them to a freezing container.

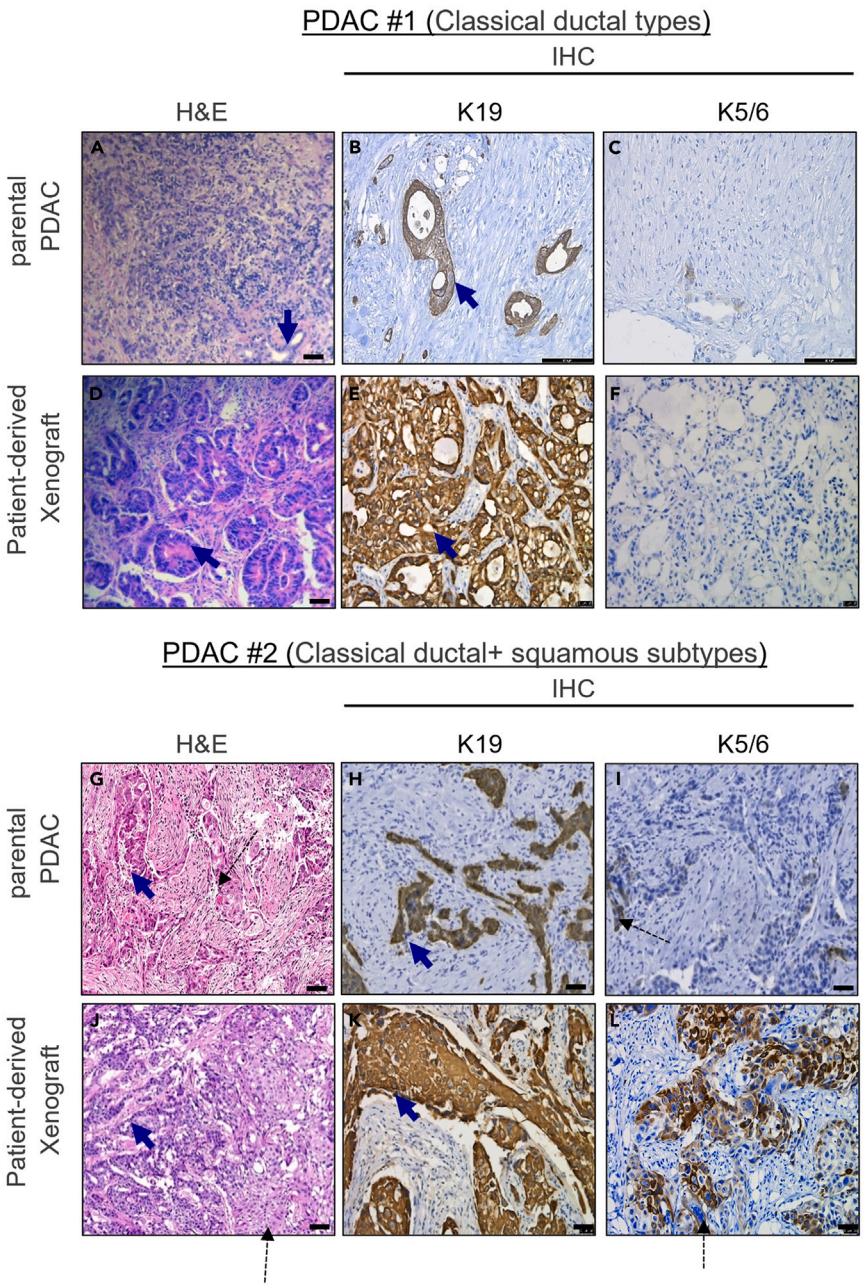


Figure 2. Representative histology images of parental and patient-derived xenograft (PDX) tumors

This figure demonstrates the first step in validating PDX tumors through histological analysis.

(A–F). Histological images of classical PDAC types. Hematoxylin and eosin (H&E) staining is shown in panels (A and D). Expression of the pancreatic ductal marker K19 is shown in panels (B and E), and the squamous or basal marker K5/6 in panels (C and F). Tumors diagnosed as moderately to poorly differentiated retained their histological features in PDX models.

(G–L) Histological images of squamous or basal subtype PDAC. H&E staining is shown in panels (G and J). Expression of K19 is shown in panels (H and K), while K5/6 expression is shown in panels (I and L). Similarly, tumors diagnosed as poorly differentiated with rare squamous cell types also retained their distinct cell types in PDX, as evidenced by K5/6 labeling for squamous cell carcinoma. Scale bars indicate 50 or 150 μm. The arrow indicates classical ductal cell types expressing K19, and the dashed arrow indicates squamous subtypes expressing K5/6.

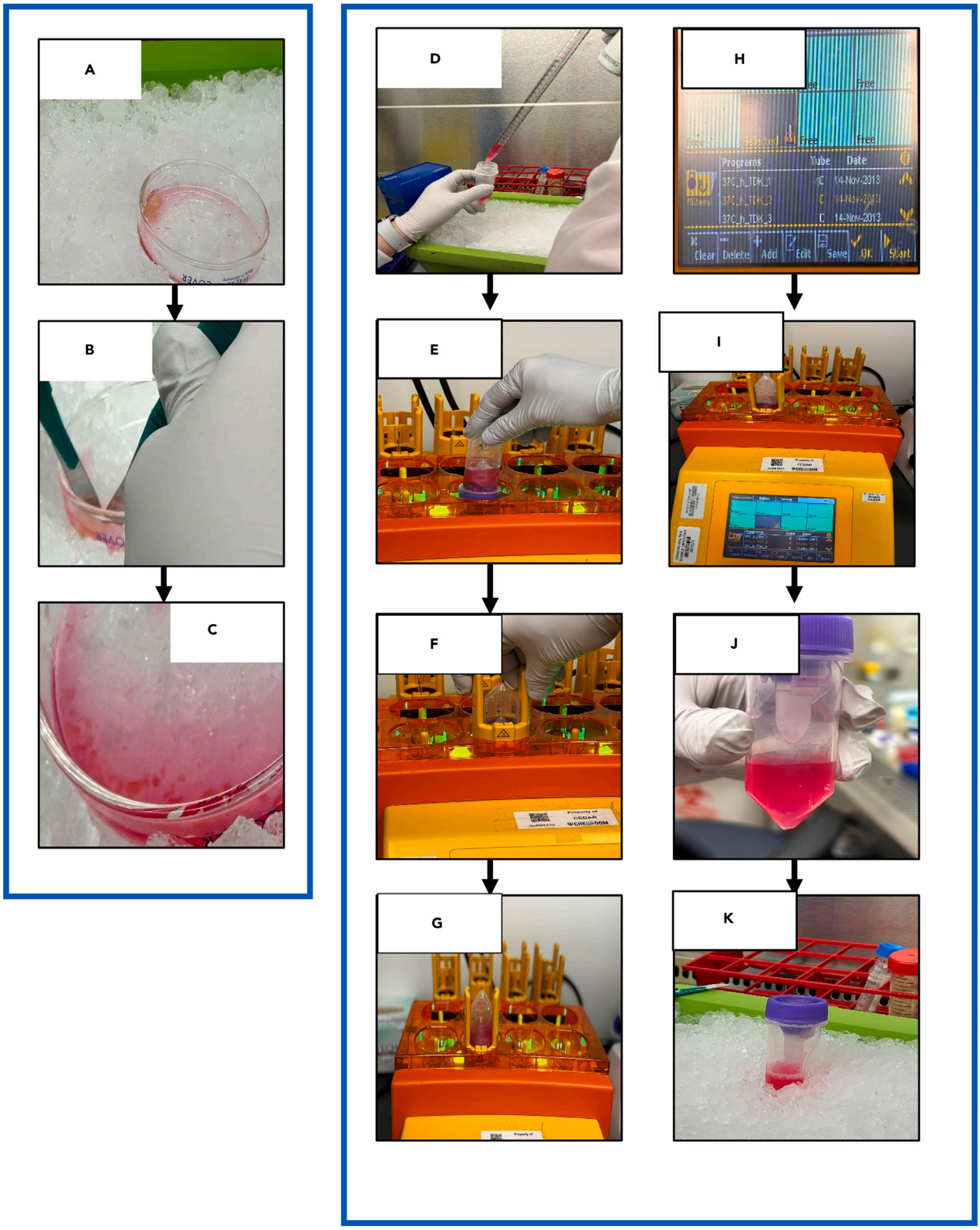

A
B
C
D
E
F
G
H
I
J
K
Programs
Tube
Date
37C_h_TDK_1
37C_h_TDK_3
14-Nov-2013
Clear
Delete
Add
Edit
Save
OK
Start

Figure 3. Schematic diagram illustrating the procedures for extracting and dissociating PDX tumors

(A) Place the tumor piece in a dish.

(B and C) Mince the tumor piece into smaller fragments.

(D) Transfer the minced tumor pieces into a gentleMACS C Tube.

(E–G) Securely attach the tightly sealed gentleMACS C Tube upside-down onto the sleeve of the gentleMACS Dissociator and place the heaters on top.

(H) Select the '37_h_TDK_2' protocol as one of the standard tumor dissociation programs.

(I) Perform the dissociation process.

(J and K) Collect the dissociated cells after completion.

vi. Keep at −80°C for 12–16 h, then store in LN2 for later use.

vii. Transplant remaining pieces of tumors into new NSG mice (see step 11).

11. Implant PDX into new NSG mice.

    a. Recover frozen tumors from the LN2 tank by rapidly thawing vials at 37°C if it starts with frozen tumors. Otherwise, see step c.

    b. Place on ice until use.

    c. Proceed with the procedure as outlined in step 3.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
TRA-1-85 PE (CD147) (1/25 dilution)	BD Biosciences	Cat#563021
TRA-1-85 BV421 (CD147) (1/25 dilution)	BD Biosciences	Cat#563302
Chemicals, peptides, and recombinant proteins		
F-12 Ham	Thermo Fisher Scientific	Cat# 11765054
Sodium bicarbonate 7.5% solution	Thermo Fisher Scientific	Cat #25080-094
HyClone amphotericin B (Fungiezone) solution	Thermo Fisher Scientific	Cat# SV3007801
Gentamycin sulfate	Thermo Fisher Scientific	Cat# 15750-060
Ciprofloxacin	Thermo Fisher Scientific	Cat #MT-61-277-RG
HBSS (10×), no calcium, no magnesium, no phenol red	Thermo Fisher Scientific	Cat #14185052
HEPES (1 M)	Thermo Fisher Scientific	Cat #15630080
DMEM/F12	Invitrogen	Cat #11330-032
High-glucose DMEM	Invitrogen	Cat # 11995
HyClone fetal bovine serum (heat-inactivated)	Thermo Fisher Scientific	Cat #SH30071.03HI
Defined K-SFM	Invitrogen	Cat #10744-019
Human EGF	BD Biosciences	Cat #354052
Cholera toxin	Sigma-Aldrich	Cat #C8052
Bovine pituitary extract	Invitrogen	Cat #13028014
Penicillin-streptomycin (10,000 U/mL)	Invitrogen	Cat #15140122
Human TruStain FcX	BioLegend	Cat #422302
Matrigel basement membrane matrix	Corning (Thermo Fisher Scientific)	Cat #354234
Collagen I, high concentration, rat tail, 100 mg	Corning (Thermo Fisher Scientific)	Cat #CB-40236 #354249
Accutase cell detachment solution	Innovative Cell Technologies	AT104
Acetic acid, glacial (certified ACS plus)	Fisher BioReagents	BP2401-500
Critical commercial assays		
gentleMACS Octo dissociator with heaters	Miltenyi Biotec	Cat #130-096-427
gentleMACS C tubes	Miltenyi Biotec	Cat #130-093-237
gentleMACS SmartStrainers 70 um	Miltenyi Biotec	Cat #130-098-462
QuadroMACS separator	Miltenyi Biotec	Cat #130-090-976
LS columns	Miltenyi Biotec	Cat #130-042-401
Tumor Dissociation Kit - Human	Miltenyi Biotec	Cat #130-095-929
Mouse cell depletion kit	Miltenyi Biotec	Cat #130-104-694
ddPCR KRAS G12/G13 screening kit	Bio-Rad	Cat #1863506
KRAS G12V reference standard	Horizon Inspired Cell Solutions	Cat #HD289
Experimental models: Organisms/strains		
Mouse: NOD-SCID-IL2Rgc null (NSG) (4–6 weeks old, female/male)	The Jackson Laboratory	JAX: #005557

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Forward primer for KRAS	IDT	AGCGTCGATGGAGGAGTTTG
Reverse primer for KRAS	IDT	TGGTCCTGCACCAGTAATATGC
Other		
Nalgene sterile disposable filter units with PES membrane (0.2–75 mm)	Thermo Fisher Scientific	Cat #566-0020
Surface treated tissue culture dishes	Fisher Scientific	Cat #FB012924
Purdue Products Betadine topical microbicide solution	Fisher Scientific	19-027136
Finger loop ear punch – 2 mm diameter	FST	Cat #24212-0
Fine scissors – tungsten carbide	FST	Cat #14568-12
Halsted-mosquito hemostats	FST	Cat #13009-12
Graefe forceps	FST	Cat #11051-10
Strabismus scissors – tungsten carbide	FST	Cat #14575-11
Ethicon suture 5-0 PDS II violet 18″ P-3 cutting	Ethicon	Cat #Z463G

MATERIALS AND EQUIPMENT

Transport media

Reagent	Storage	Stock conc.	Final conc.	Volume
Sodium Bicarbonate 7.5% solution	4°C	7.50%	0.12%	8 mL
Penicillin/Streptomycin	−20°C	100×	1×	5 mL
HyClone Amphotericin B (Fungiezone) Solution	4°C	250 μg/mL	2.5 μg/mL (1%)	5 mL
Gentamycin Sulfate	4°C	50 mg/mL	0.01 mg/mL (10 μg/mL)	0.1 mL
Ciprofloxacin	24°C	10 mg/mL	10 μg/mL	0.5 mL
F-12 Ham	4°C		1×	481 mL
Total				500 mL

Aliquot it into single-use and store at −20°C for up to 1 year.[6,7]

25 mM HEPES/HBSS buffer

Reagent	Storage	Stock conc.	Final conc.	Volume
Hank's balanced buffered saline solution (HBSS)	4°C	10×	1×	10 mL
HEPES 1 M	4°C	1 M	25 mM	2.5 mL
Sterile Water	24°C	N/A	N/A	87.5 mL
Total			**N/A**	**100 mL**

Store at 4°C for up to 3 months.

Human PDAC media

Reagent	Storage	Stock conc.	Final conc.	Volume
Human EGF	−80°C	10 μg/mL	5 ng/mL	250 μL
Cholera Toxin	4°C	1 mg/mL	50 ng/mL	25 μL
Bovine Pituitary Extract	−20°C	15.3 mg/mL	50 μg/mL	1634 μL
Pen/Strep	−20°C	10,000 U/mL	1%	5 mL
Completed Defined K-SFM	4°C	N/A	N/A	493 mL
Total			N/A	500 mL

Store at 4°C for up to 3 months.[6,7]

Collagen working solution

Reagent	Storage	Stock conc.	Final conc.	Volume
Acetic Acid	24°C	0.02 N	0.02 N	19.579 mL
Rat Tail Collagen	4°C	3.52 mg/mL	74 μg/mL	421 μL
Total			N/A	20 mL

Freshly make the solution.

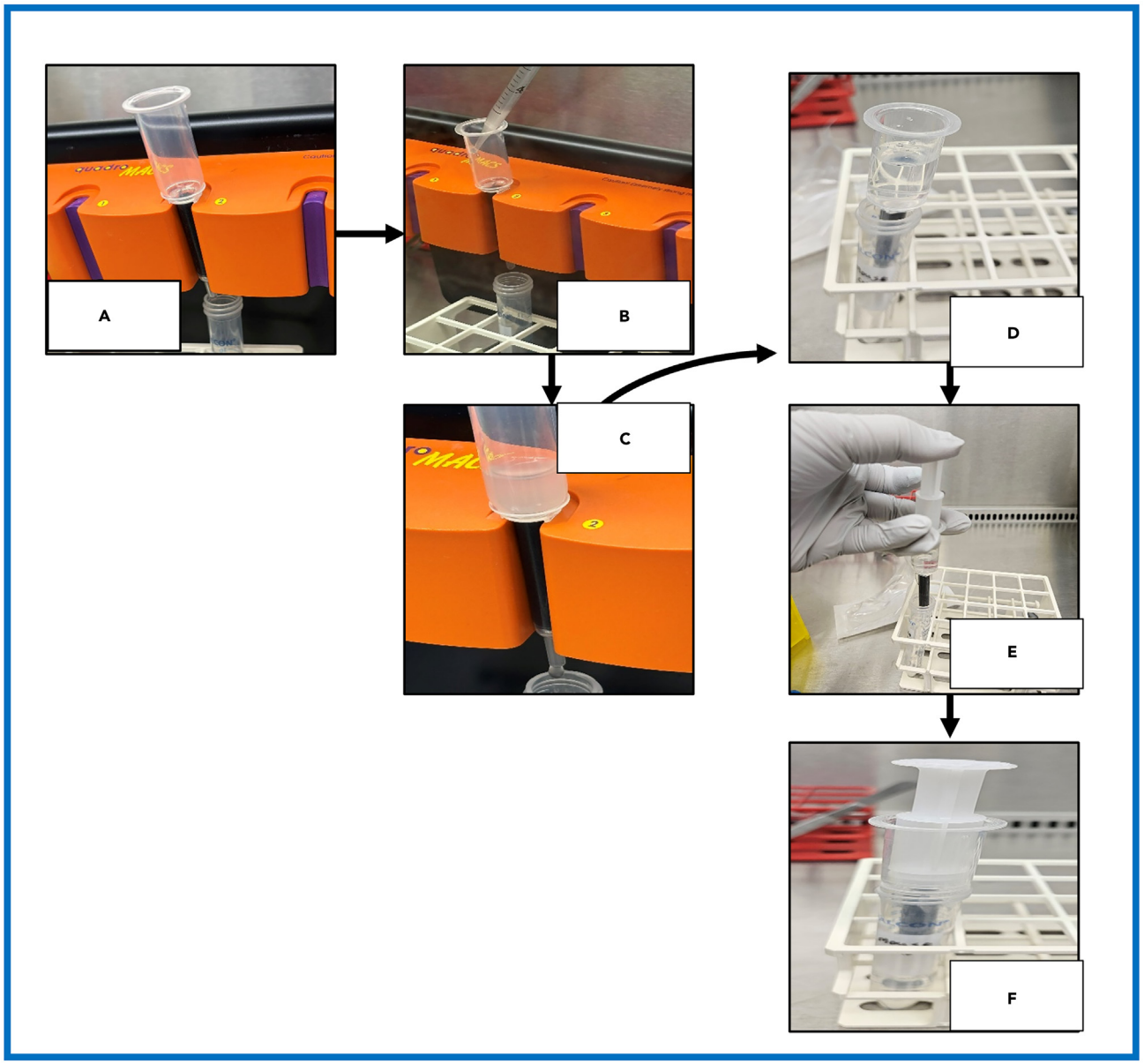


Figure 4. Schematic diagram illustrating the procedure for depleting mouse cells using a MACS column

(A) Equilibrate the MACS column with MACS buffer and discard the eluate into a waste tube.

(B and C) Load the dissociated PDAC cells into the equilibrated column and collect the flow-through containing human PDAC cells.

(D–F) Remove the MACS column from the magnetic field and flush out the magnetically labeled mouse cells using a plunger to collect the bound fraction containing mouse cells.

STEP-BY-STEP METHOD DETAILS

Extract and dissociate PDX tumors

Timing: 4 h; see the graphical abstract for the schematic diagram

⚠ CRITICAL: The size of the tumor pieces to be transplanted determines the growth rate. Too small tumor pieces take approximately three months to grow after transplantation. However, tumors larger than 1 cm^3 often develop necrosis, which can compromise the

quality of the sample. Therefore, euthanize the NSG mice when the tumor reaches a volume of 1 cm^3, but do not allow the tumor to grow beyond this size.

1. Extract PDX tumors from NSG mice.
   a. Label glass vials, add 10 mL plain DMEM without FBS or antibiotics, and keep them on ice.
   b. Anesthetize the NSG mice using isoflurane (see step 3 before you begin).
   c. Extract and transfer the tumor to DMEM in the cold glass vial.
   d. Euthanize the mouse by cervical dislocation and transfer tissue to a glass dish on ice under the TC hood.
   e. Save a portion of the tumor piece for H&E staining from each mouse in 4% PFA and store at 4°C until use.
2. Mincing tumor pieces. (Figure 3) (See problem 2).

⚠ CRITICAL: Tissue often adheres to plastic surfaces, so use a glass dish for the dissociation process. Always keep the tumor pieces on ice to minimize unwanted cell death and complete the dissociation process as quickly as possible.

   a. Remove fat, fibrous and necrotic areas from the tumor sample on ice.
   b. Wash tumors with fresh DMEM once and then measure weight.
   c. For each tumor, transfer 1 g of tumors in a glass vial containing 10 mL of fresh DMEM and keep on ice until ready to use.

Note: A tumor size of 1 g is optimal for achieving the best dissociation yield. If the tumor exceeds 1 g, divide it into 1 g portions for processing (refer to Problem 2).

   d. Transfer 1 g of tumor in a glass dish containing 1 mL of tumor dissociation kit solution in step 3.a. ii.
   e. Mince each tumor into small pieces (no larger than 1–2 mm^3) using scissors or a blade on ice.

Note: Using a smaller volume of dissociation solution improves the efficiency of mincing. After mincing is complete, add 9 mL of tumor dissociation solution back to the sample and keep it on ice.

3. Dissociate PDX tumors using gentleMACS Dissociator (Figure 3, See problem 3).
   a. Prepare tissue dissociation kit solution.
      i. Make aliquots of Enzyme H, R and A and store them at −20°C.

Enzyme H	3 mL of DMEM	Aliquot 1600 µL and store −20°C	Stable at −20°C for 6 months
Enzyme R	2.7 mL of DMEM	Aliquot 800 µL and store −20°C	Stable at −20°C for 6 months
Enzyme A	1 mL of Buffer A	Aliquot 100 µL and store −20°C	Stable at −20°C for 6 months

      ii. GentleMACS can only handle eight gentleMACS C tubes, each one of which can handle 1g of tumor. On the day of the tumor dissociation, mix the following according to the amount of tumor you have.

Components	For 1 MACS C tube/1 g tumor
DMEM	9.4 mL
Enzyme H	400 µL
Enzyme R	200 µL
Enzyme A	50 µL

      iii. Keep on ice until use.

b. Transfer each dish's contents into a gentleMACS C tube using a 25-mL pipet (1 g max of tumor per tube). (Figure 3).

Note: If the tissue pieces do not pass through the opening of a 25-mL pipette, continue mincing until they are small enough to fit.

c. Tightly close the C tubes and attach them upside down onto the sleeve of the gentleMACS Dissociator. Then, place the heaters on top of them.
d. Run the 37_h_TDK_237C_h_TDK_2 dissociation program (Duration 1 h and 1934 rotations per revolution: RPR) on the gentleMACS Dissociator.

Note: This program is designed specifically for medium tumor tissue types, depending on the tissue's histological composition (Figure 3). The concentration of the Miltenyi enzyme cocktails was adjusted based on the size of the tumor.

i. After the program terminates, detach the C tubes from the gentleMACS Dissociator and return the tumor solution to ice.

Note: For procedures requiring single-cell dissociation, complete the full digestion program to ensure the tumors are fully dissociated, achieving a consistency similar to rice slurry. Alternatively, for culturing cells, the presence of cell clumps can enhance survival. To facilitate this, perform partial digestion by reducing the enzyme concentrations (Figure 3).

4. Wash dissociated tumors.

Note: After tumor dissociation, thoroughly wash the dissociated tumor cells with an appropriate buffer to eliminate any residual debris and ensure a clean cell preparation for subsequent processing.

a. Apply cell suspension to 70 μM MACS strainer.
b. Apply an equal volume of quenching buffer on a strainer and collect in the same previously used tube to inactivate enzymes.
c. Rinse the C-tube and lid with 10 mL of quenching buffer and apply it to the previous strainer to collect in the same 50 mL tube.
d. Combine and spin down cells at 300 g at 4°C for 5 min and then decant cloudy supernatant.
e. Loosen the pellet by tapping or flicking the tubes (do not vortex) and resuspend it by pipetting up and down with 10 mL of MACS Buffer.
f. Repeat step 4c and combine respective tumors.
g. Add MACS buffer to add the final volume of up to 50 mL.
h. Count cells using Trypan Blue 1:1 and Cell Counter.
i. Set aside 1/5 of the dissociated cells in a 15mL falcon tube labeled as "before mouse cell depletion. This sample will serve as a control for subsequent flow cytometry analysis. Centrifuge the saved sample at 300 g for 5 min, then carefully decant the supernatant and keep it on ice until ready for flow cytometry.

Mouse cell depletion

Timing: 1 h (Figure 4: schematic diagram, see problem 4).

Deplete mouse cells using a mouse cell depletion kit with magnetic-activated cell sorting (MACS). This process ensures the removal of contaminating mouse cells, enabling the enrichment of human PDAC cells in the culture.

5. Loosen pellet and stain cells with Mouse Cell Depletion Cocktail from Miltenyi following the chart.

Note: Use reagent volumes appropriate for 1 g of tumor. If starting with 2 g of tumor, split the sample into two portions and process each separately.

Tumor	1 g
MACS buffer	160 μL
Mouse Cell Depletion Cocktail	40 μL

6. Mix and incubate for 10 min at 4°C in the dark.
7. Equilibrate the MACS columns with MACS buffers.
   a. Set up the MACS column and separator in the TC hood during incubation.
   b. Label three 15 mL conical tubes: i) Waste, ii) flow-through (Human PDAC), iii) bound (Mouse cells) for each condition.
   c. Equilibrate the column by adding 3 mL of MACS buffer, allowing it to pass through to the "waste" conical by gravity flow and discard 'waste' tubes.
   d. Place a "human PDAC" conical under each column.
8. Load the cell resuspension into the column carefully to allow mouse cells to attach properly to the column.

Note: This process enables the collection of human PDAC cells.

   a. Resuspend cells in 2.8 mL of MACS buffer.
   b. Transfer them onto the column.
   c. Collect "flow-through fractions (human PDAC)."
   d. Wash the column twice with 3 mL of MACS buffer (collect in the same tube).
9. Retrieve mouse cells from the column after the separation process.

Note: This population can be used for mouse CAF cell culture and assessed for the efficiency of mouse cell depletion. See step 20.

   a. Detach columns and place them on top of the "bound fractions (mouse cells)" conical tube.
   b. Add 3 mL of MACS buffer per column.
   c. Fush out magnetically labeled mouse cells using a plunger.
10. Count the number of collected cells to determine the total cell yield.
   a. After separation, combine all "flow-through (hPDAC) fraction" tubes (9 mL x 4 and add MACS buffer to bring the final volume of cells to 50 mL) and all "bound (mouse cell) fraction" tubes (3 mL x4 = 12 mL).
   b. Determine the number of cells using a cell counter.
11. Centrifuge all three fractions at 500 g for 5 min at 4°C and aspirate supernatant.
12. Resuspend the cell pellet in 5–10 mL of Complete human PDAC media.

Note: Adjust the volume of media depending on the size of the pellet.

   a. Divide the cell suspension aliquots from each fraction for the following purposes.
   b. For TRA-1-85 staining, dilute cells with media to make a concentration of 1×10^6 cells/mL.

Note: For flow cytometry for TRA-1-85 staining, 1×10^6 cells for TRA-1-85 staining are required for two antibodies (4-5 × 10^5 cells/one antibody).

      i. Aliquot 400 μL of cells from each fraction (two tubes: Unstained and TRA-1-85 PE or TRA-185 BV421).

ii. Spin down cells and proceed to step 13.

c. For genomic DNA extraction to be used for verifying KRAS mutation, harvest 1 × 10^5 cells (=~3 μg total DNA) for genomic DNA extraction. Proceed with step 14.

d. For RNA extraction for RNA-seq, dilute cells to make 1 × 10^6 cells/mL.

Note: 1 × 10^6 cells are required for each sample for RNA extraction for RNA-seq.

i. Spin down and store the cell pellet at −80°C until use.

e. For culture: proceed to step19.

Verification of human cell population by flow cytometry

Timing: 2–3 h (Figure 5A: schematic diagram).

To verify the efficiency of mouse cell depletion, we analyze the cells using the TRA-1-85 human-specific marker via flow cytometry before and after the depletion process. This will allow for a comparison of mouse and human cell populations, confirming the success of the depletion.

13. Prepare cells for TRA-1-85 staining for flow cytometry.
    a. Block cells with FcR blocker.
        i. Dilute the FcX solution at a 1:50 ratio in PBS.
        ii. Resuspend 100 μL of the 1/50-diluted FcR blocker with cells (4–5 × 10^5 cells per tube) prepared in step 12b.
        iii. Incubate the mixture for 10 min at 24°C, ensuring it is protected from light.
    b. Stain cells with TRA-1-85.
        i. Dilute TRA-1-85 antibodies (PE and BV421) 1/25 in PBS.
        ii. Add diluted TRA-1-85 antibodies to 100 μL of cells in the FcR blocker.
            -TRA-1-85 BV421: 100 μL of 1/25 diluted TRA-1-85 BV421.
            -TRA-1-85 PE: 100 μL of 1/25 diluted TRA-1-85 PE.
    c. Add 100 μL of PBS in unstained control cells.
    d. Incubate for 30–45 min at 4°C.
    e. Add 2 mL PBS, spin 300 g for 5 min at 4°C and decant.
    f. Resuspend cells in 300 μL of 4% PFA and store at 4°C.
    g. Load samples and acquire data on BD Symphony (Figure 5B).

Verification of PDAC harboring KRAS mutations

Timing: 2–3 h (Figure 5A: schematic diagram).

To verify the presence of PDAC cells in dissociated tumors enriched after mouse cell depletion, we analyze the fraction of cells harboring common oncogenic KRAS mutations (e.g., KRAS G12D, G12R, G12V) using digital droplet PCR (ddPCR). If the specific KRAS mutation in the patient is unknown, Sanger sequencing is performed to identify the mutation.

14. Prepare cells for genomic DNA extraction.
    a. Aliquot 200 μL of cells from each fraction in the 1.5 mL tube and keep it on ice.
    b. Spin cells at 20800 RCF (14000 rpm) for 30 s (1 min if you cannot see any pellet).
    c. Rapidly decant the media and centrifuge the cells one more time.
    d. Carefully pipe out the residual media with a 20 or 200 μL tip.
    e. Snap freeze cell pellet at −80°C until genomic DNA is isolated.
    f. Isolate genomic DNA from the cells using either a commercially available kit, following the manufacturer's protocol, or manually, depending on your specific experimental preference.

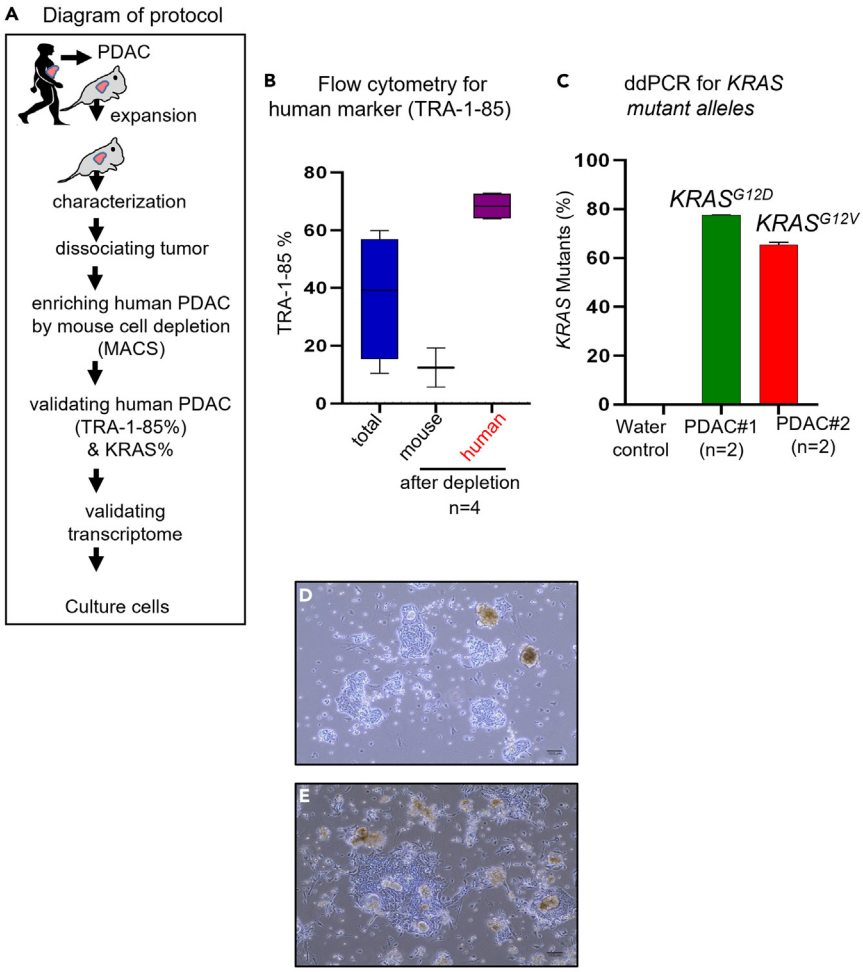


Figure 5. Enrichment of human PDAC harboring KRAS mutations from dissociated PDAC tumors

(A) Schematic diagram illustrating the protocol for dissociating and enriching human PDAC cells from patient-derived tumor samples.

(B) Flow cytometry analysis using the TRA-1-85 human cell surface marker to confirm the enrichment of human PDAC cells.

(C) Droplet digital PCR (ddPCR) results show the presence of KRAS mutations in the enriched human cell population.

(D and E) Representative images of two PDAC cells 72 h after plating, illustrating cell morphology and attachment. The scale bar indicates 100 μM.

g. Store purified genomic DNA at 4°C until use (go to steps 15–16 for KRAS verification).

15. Detection of point mutations by ddPCR

Note: ddPCR can handle a maximum of 60 ng of DNA per well. To analyze genomic DNA using ddPCR, prepare a 10 μL solution with a concentration of 2.5 ng/μL. As ddPCR is highly sensitive to salts, ensure that only ultrapure (UP) H_2O is used for DNA dilution.

a. Prepare the following reaction for each sample in a well of a 96-well plate.

Note: Spin down the Supermix and Probe before use to ensure proper mixing and avoid any inconsistencies in the assay.

PCR reaction master mix	
Reagent	Amount (μL)
DNA template (2.5 ng/μL)	9. 9
20× SuperMix	1.1
20× primer probe	1.1
ddH_2O	9.9
Total	22

b. Mix with gentle pipetting (avoid air bubbles at all costs), seal the plate and spin it down.
c. Use QX200 AutoDG to generate droplets.

Note: Fill the empty "sample" wells with water to maintain balance and consistency in the assay.

d. Seal the new plate and run the following program "DDPCR_KRAS" total volume: 40 μL, Lid temperature: 105°C, Ramp: 2°C/s

DDPCR_KRAS cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	10 min	1
Denaturation	94°C	30 s	39 cycles
Annealing/Extension	55°C	1 min	
Inactivation	98°C	10 min	1
Hold	4°C	forever	

e. Turn on the Computer / Login / Droplet Reader / Software in this order.
f. Open the QuantaSoft software desktop shortcut named "use for ddPCR" and set up the template for ddPCR droplet reader.

Note: The template can be prepared in advance for convenience.

i. Highlight wells with samples, then hit Enter.
ii. Set the experiment parameter as ABS (absolute quantification).
iii. Define Supermix with Supermix without dUTPs.
iv. Tagged 1 (channel 1): KRAS mutant (FAM) / Type: Ch.1 unknown.
v. Tagged 2 (channel 2): KRAS WT (HEX) / Type: Ch. 2 unknown.
vi. To add sample names, double-click well, type information, and hit Enter.
vii. Save the template.
viii. Insert the plate into the droplet reader and check that the corresponding green light is on.

g. Load the template and run (Figure 5C).

16. Perform PCR amplification of the region of interest to prepare the sample for sequencing. Subsequently, verify the presence of the KRAS mutation through sequencing.
a. Set up the following reaction in a PCR microcentrifuge tube on ice.

PCR reaction master mix	
Reagent	Amount (μL)
DNA template	variable (50 ng total)
Taq DNA Polymerase	0.25
10 μM Forward Primer	1
10 μM Reverse Primer	1
10× Standard Taq Reaction Buffer	5
10 mM dNTPs	1
Ultra-Pure H_2O	Up to 50 μL

b. Prepare a master mix to add on top of your template DNA and add a water control with your samples.
c. Gently mix the reaction and transfer PCR tubes from ice to a PCR machine with the block pre-heated to 95°C and begin thermocycling.

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	30 s	30cycles
Annealing	55°C	30 s	
Extension	68°C	30 s	
Final extension	68°C	5 min	1
Hold	4°C	forever	

d. Submit Unpurified PCR products for Sanger sequencing with forward and revere primers.

Culturing human PDAC cells

Timing: 1 h (Figures 5D–5E, see problem 5).

Herein, we will describe a protocol for culturing PDAC cells in a monolayer format.

17. Prepare the human PDAC medium according to the instructions provided in the materials and equipment Setup section. Ensure all components are mixed thoroughly, and the medium is sterile before use.
    a. Mix the entire vial (1 mL) of [Defined Keratinocyte-SFM Growth Supplement] into a 500mL bottle of [Defined Keratinocyte-SFM] media.
    b. Add the following components to 493 mL of [Defined K-SFM media + Supplement: 250 µL of Human rEGF (Stock conc. = 10 µg/mL), 25 µL of Cholera Toxin (Stock conc. = 1 mg/mL), 1634 µL of Bovine Pituitary Extract (Stock conc. = 15.3 mg/mL), 5 mL of Penicillin-Streptomycin (Stock conc. = 10 µg/mL)
    c. Filter Media through 0.2 µm Sterile filer unit with PES membrane.
18. Prepare 10 µg/cm^2 collagen-coated plates. a. Mix 109 µL of Rat Tail Collagen with 5.9 mL of 0.02 N acetic acid to make 100ug/cm^2 collagen solution (Stock conc. = 4.42 mg/mL) and store at 4°C.

Note: Stock concentrations may vary between lots. Based on the specific stock concentration of the current lot, recalculate the required volume to achieve the final concentration.

    a. Incubate at 37°C in a 5% CO_2 incubator for 2 h or 12–16 h.
    b. Aspirate the collagen solution out and rinse with plain DMEM three times.

⚠ CRITICAL: Rinse the dish thoroughly multiple times (at least three times) to ensure complete removal of the acetic acid solution as it dissolves collagen.

    c. After the final wash, add a small volume of PDAC medium to equilibrate the plate.
    d. Return it to the 5% O_2, 5% CO_2, 37°C incubator until use.
19. Centrifuge the 'flow-through (human cell) fraction' tubes from Step 12 at 300 g for 5 min at 4°C and aspirate the supernatant.
20. Resuspend cell pellets in PDAC medium to achieve a plating density of 1 × 10^5 cell/cm^2 (optional). To derive human CAF cells from PDAC tumors, use freshly resected tumor tissue and dissociate it as described in steps 13–15. To derive mouse CAF cells, use retrieved mouse cells from Step 9. Then, culture the cells on gelatin-coated dishes in DMEM supplemented with 10% FBS.

21. Transfer the appropriate volume of resuspended cells into dishes and add PDAC medium to the final volume based on dish size.
22. Return the dish to the 5% O_2, 5% CO_2, 37°C incubator.
23. Change the PDAC medium 16 h after initial plating, then every 48 h thereafter. 24. Use the cultures for specific purposes, such as gene delivery[1] or drug screening.[8]

EXPECTED OUTCOMES

Patient-derived xenograft (PDX) models of pancreatic ductal adenocarcinoma (PDAC) closely resemble their parental tumors, faithfully retaining key histological features. Tumors initially diagnosed as moderately or poorly differentiated maintain their characteristics in PDX models, while those diagnosed as moderately differentiated with squamous subtypes retain their distinct cell types, as demonstrated by K5/6 labeling for squamous or basal subtypes (Figure 2). Importantly, aggressive tumor subtypes, such as squamous or basal types, become enriched in PDX models over time. This protocol is especially useful for isolating and culturing rare cell populations within PDAC tissues, such as basal and squamous subtypes, which typically make up less than 5% of the parental tumor. However, these subtypes become more prevalent as PDX tumors are serially passaged in NSG mice (Figure 2).

The time required for tumor formation depends on the size and condition of the original tumor, with the initial engraftment period lasting 3–4 months. Successive passages reduce this duration as human stromal cells are depleted.[9,10] Approximately 1–2 million human PDAC cells can be harvested from 1 g of PDX tumor tissue. After mouse cell depletion, around 70–90% of the remaining cells will be of human origin, carrying KRAS mutations (Figure 5). This method is highly advantageous for culturing purified PDAC cells, enabling efficient transduction with ectopic genes to manipulate gene expression and perform drug screening.

LIMITATIONS

The protocol described herein is designed to enrich and expand human PDAC cells while depleting CAF cells. As a result, it does not account for the influence of the tumor microenvironment on PDAC in response to specific drugs or gene expression changes. To better capture tumor heterogeneity, additional steps—such as co-culturing PDAC cells with isolated human CAF—are required. Human CAF cells in PDX tumors are replaced by mouse fibroblasts over time.[9,10] Accordingly, a portion of the freshly resected tumor from patients should be set aside for culturing CAF cells, which can then be derived following the procedure outlined in Step 20b.

TROUBLESHOOTING

Problem 1

Low success rate of PDX transplantation (related to Step 4 in section: implantation of human PDAC into NOD-SCID IL2Rgammanull (NSG) mice in before you begin).

Potential solutions

- While the condition of the parental tumor is important for transplantation efficiency, the size of the tumor fragments used also significantly influences the success rate. Smaller tumor pieces take longer to grow, while larger pieces can reduce tumor formation efficiency. To optimize transplantation success, limit the size of tumor fragments to approximately 2–3 mm^3.

Problem 2

Low yield of human PDAC cells from PDX tumors. (related to steps1-2).

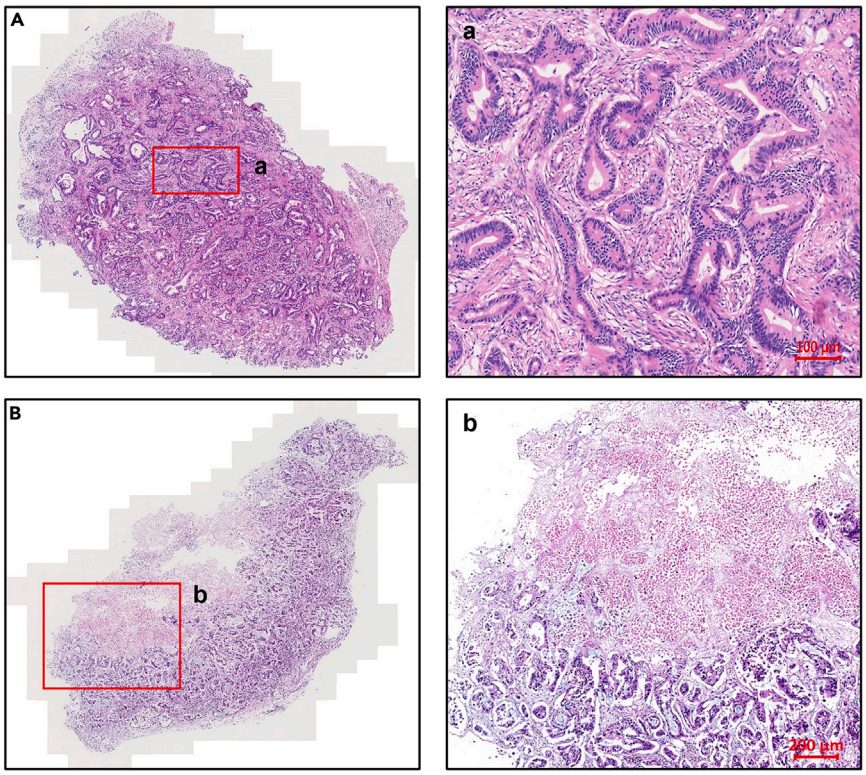


Figure 6. Representative H&E images of the necrotic area of tumors
(A) Example of a PDX tumor without necrosis (inset, a').
(B) PDX tumor with necrotic regions (inset, b').

Potential solutions

- Do not let tumors grow larger than 1 cm^3. Tumors exceeding this size generally develop necrotic regions, producing a poor yield of viable human PDAC cells (Figure 6).
- Ensure the complete removal of the necrotic area before proceeding with mincing.
- The mincing step is the most critical part of the procedure, as it directly impacts the quality of the tumor. Ensure that the initial tumor size is below 1g per reaction and that the tumor pieces are sufficiently minced, allowing smooth passage through a 25-gauge pipette (see step 1).

Problem 3

Tissue chunks remain after dissociating the PDX tumor using a gentle MACS Dissociator (Related to step 2). Once the program is finished, the suspension should be cloudy with minimal chunks. If larger chunks remain.

Potential solutions

- Remove and invert the c-tube and let it settle on ice for 5 min.
- Remove 9 mL of supernatant, leaving about 1 mL of media and tumor chunks.
- Place C-tube back on gentleMACS Dissociator.
- Run the m_imptumor_01 program once, which is specifically designed for the gentle dissociation of soft to medium mouse tumors.
- Combine the resulting cell suspension with the previously removed supernatant and resuspend.

Problem 4

Low efficiency in mouse cell depletion or clogging of the MACS column can occur during the process. (related to step 5–12).

Potential solutions

- **Avoid cell aggregation** before incubating with mouse cell marker cocktails and passing the sample through the MACS column. Ensure the cell suspension is well-dissociated and filtered to remove clumps.
- **Optimize cell numbers:** Each LS MACS column can technically handle 1 × 10^8 labeled mouse cells and 2 × 10^9 total cells. However, we advise using a maximum of 5-7 × 10^7 cells per column. Since 1 g of PDX tumor typically yields approximately 50 million cells, use 1 g tumor per column and limit the number of cells loaded into each MACS column to match its maximum capacity for optimal performance.

Problem 5

Cells do not attach or grow out after 72 h of plating (related to steps 17–20).

Potential solutions

- **Ensure optimal conditions**: Confirm that plating density, medium composition, and substrate coating are appropriate for the cell type.
- **Avoid overdigesting:** Overdigesting can compromise cell quality. Optimize the digestion process by adjusting enzyme concentration and digestion time.
- **Basal subtype PDAC**: Cells of the basal subtype can easily attach to plates, even as single cells.
- **Classical PDAC**: For classical PDAC, leaving small cell clumps can facilitate attachment and growth. If single cells must be plated, ensure a higher plating density for better outcomes.
- **Floating cells**: Re-plate any floating cells onto a new coated plate, as they tend to delay attachment but can still adhere and grow successfully.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact: Jungsun Kim (kimjungs@ohsu.edu).

Technical contact

Questions about the technical specifics of performing the protocol should be directed to and will be answered by the technical contacts: Julien Tessier (tessierj1987@gmail.com), Dmytro Grygoryev (grygorye@ohsu.edu), Marilynn Chow-Castro (chowcast@ohsu.edu).

Materials availability

PDX tumors generated in this study are available upon request. This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyze datasets or code.

ACKNOWLEDGMENTS

We thank the Brenden-Colson Center for Pancreatic Care, the Oregon Pancreas Tissue Registry, the OHSU Knight BioLibrary, and the Molecular Virology Core at the Oregon National Primate Research Center (NIH P51OD011092). The IRB protocol is the Oregon Pancreas Tissue Registry (IRB #3609). The work was funded by a CRUK-OHSU Project Award (C65925/A26986 to J.K.), MRF New Investigator Grant (GCNCR1042A to J.K.), and Knight CEDAR (68182-933-000 and 68182-939-000) from the Cancer Early Detection Advanced Research Center at OHSU Knight Cancer Institute. Graphical abstracts and figures were created using Biorender.com.

AUTHOR CONTRIBUTIONS

J.T.: writing – original draft, methodology development, data acquisition, and formal analysis. D.G.: writing – review and editing, investigation, data acquisition, and formal analysis. M.C.-C.: methodology development, data acquisition, and formal analysis. T.E., S.-W.L., and M.B.: investigation, methodology, and data acquisition. E.M.: resources, methodology, and investigation. J.M.L., D.K., and B.L.A.-P.: resources and methodology. B.S.: resources. T.M.: resources and formal analysis. R.C.S.: supervision and resources. J.K.: writing – review and editing, writing – original draft, supervision, project administration, investigation, funding acquisition, and formal analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Grygoryev, D., Ekstrom, T., Manalo, E., Link, J.M., Alshaikh, A., Keith, D., Allen-Petersen, B.L., Sheppard, B., Morgan, T., Soufi, A., et al. (2024). Sendai virus is robust and consistent in delivering genes into human pancreatic cancer cells. Heliyon *10*, e27221. https://doi.org/10.1016/j.heliyon.2024.e27221.

2. Biffi, G., and Tuveson, D.A. (2021). Diversity and Biology of Cancer-Associated Fibroblasts. Physiol. Rev. *101*, 147–176. https://doi.org/10.1152/physrev.00048.2019.

3. Biankin, A.V., Waddell, N., Kassahn, K.S., Gingras, M.C., Muthuswamy, L.B., Johns, A.L., Miller, D.K., Wilson, P.J., Patch, A.M., Wu, J., et al. (2012). Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature *491*, 399–405. https://doi.org/10.1038/nature11547.

4. Martinez-Sabadell, A., Ovejero Romero, P., Arribas, J., and Arenas, E.J. (2022). Protocol to generate a patient derived xenograft model of acquired resistance to immunotherapy in humanized mice. STAR Protoc. *3*, 101712. https://doi.org/10.1016/j.xpro.2022.101712.

5. Ben-David, U., Ha, G., Tseng, Y.Y., Greenwald, N.F., Oh, C., Shih, J., McFarland, J.M., Wong, B., Boehm, J.S., Beroukhim, R., and Golub, T.R. (2017). Patient-derived xenografts undergo mouse-specific tumor evolution. Nat. Genet. *49*, 1567–1575. https://doi.org/10.1038/ng.3967.

6. Kim, J., and Zaret, K.S. (2019). Generation of Induced Pluripotent Stem Cell-Like Lines from Human Pancreatic Ductal Adenocarcinoma. In Pancreatic Cancer: Methods and Protocols, G.H. Su, ed. (New York: Springer), pp. 33–53. https://doi.org/10.1007/978-1-4939-8879-2_4.

7. Kim, J., Hoffman, J.P., Alpaugh, R.K., Rhim, A.D., Reichert, M., Stanger, B.Z., Furth, E.E., Sepulveda, A.R., Yuan, C.X., Won, K.J., et al. (2013). An iPSC Line from Human Pancreatic Ductal Adenocarcinoma Undergoes Early to Invasive Stages of Pancreatic Cancer Progression. Cell Rep. *3*, 2088–2099. https://doi.org/10.1016/j.celrep.2013.05.036.

8. Hirt, C.K., Booij, T.H., Grob, L., Simmler, P., Toussaint, N.C., Keller, D., Taube, D., Ludwig, V., Goryachkin, A., Pauli, C., et al. (2022). Drug screening and genome editing in human pancreatic cancer organoids identifies drug-gene interactions and candidates for off-label treatment. Cell Genom. *2*, 100095. https://doi.org/10.1016/j.xgen.2022.100095.

9. Yoshida, G.J. (2020). Applications of patient-derived tumor xenograft models and tumor organoids. J. Hematol. Oncol. *13*, 4. https://doi.org/10.1186/s13045-019-0829-z.

10. Chao, C., Widen, S.G., Wood, T.G., Zatarain, J.R., Johnson, P., Gajjar, A., Gomez, G., Qiu, S., Thompson, J., Spratt, H., and Hellmich, M.R. (2017). Patient-derived Xenografts from Colorectal Carcinoma: A Temporal and Hierarchical Study of Murine Stromal Cell Replacement. Anticancer Res. *37*, 3405–3412. https://doi.org/10.21873/anticanres.11707.