Initiation of primary luminal breast tumor organoids from tumor scrapes

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Janes Lab Protocols

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When referring to this protocol, please cite: Przanowska RK*, Labban N*, Przanowski P, Hawes RB, Atkins KA, Showalter SL†, Janes KA†. (2024) Patient-derived response estimates from zero-passage organoids of luminal breast cancer. *Breast Cancer Res*, 26, 192.

Before starting:

- See "Clinical communication and collection of information for surgical cases" protocol for coordinating sample pickup and transfer

1. Use a scalpel to scrape tumor cells from the resected tissue and smear on six uncharged glass slides (Fisher Scientific 12550433). Place up to two slides (back-to-back) in a 50-ml conical tube containing 35 ml of adDMEM/F12+++ with 70 µl of Primocin (final concentration = 100 µg/ml) prechilled on wet ice and transfer to the lab.
   - *This step is done with the help of a technician in surgical pathology (they know to avoid dye-marked regions for scrapes).*
   - *For tissue acquisition, see protocol titled "Clinical communication and collection of information for surgical cases."*
   - *Scrapes for DNA/RNA isolation are collected on four additional charged slides (SuperFrost or equivalent; two slides for DNA, two slides for RNA) and transported in empty conical tubes without recovery medium. Dye contamination is not problematic for these samples.*
   - *A smear of tissue may also be collected directly on a charged slide for subsequent RNA FISH or immediate hematoxylin and eosin (or hemadiff) staining in surgical pathology.*
2. Centrifuge tubes at 450 rcf for 5 minutes at 8ºC.
   - *If a large amount of tissue remains on the slides, recover as much as possible by pipetting recovery medium on the slide to rinse off the cells then re-centrifuge. The rinsing will also increase the fat content of the recovered sample, so additional tube changes might be necessary.*
3. Aspirate all but 1 ml of the recovery medium, use a 1-ml tip or 5-ml serological pipette pre-wetted with D-BSA to resuspend the tissue, and combine the contents of all tubes into a single 1.5-ml centrifuge tube (S = small) or 15-ml conical tube (L = large). Centrifuge at 300 rcf (S) / 450 rcf (L) for 5 minutes at RT (S) / 8ºC (L).
   - *Choice of 1.5 ml or 15 ml tube depends on whether there is a small (S) or large (L) amount of material.*
   - *Pellets from scrapes tend to be small, so 1.5 ml tube is recommended to minimize material loss.*
4. Aspirate all but 0.2 ml (S) / 2 ml (L) of the supernatant, add 1 ml (S) / 7 ml (L) of D-BSA, and resuspend the pellet. Centrifuge at 300 rcf (S) / 450 rcf (L) for 5 minutes at RT (S) / 8ºC (L).
5. Repeat Step 4 at least one more time to dilute out potential microorganisms, contaminating cell types, and dead cells/debris.
   - If the supernatant is not clear, add additional washing steps until the supernatant is clear.
6. Aspirate all the supernatant carefully, so that only the sedimented tissue pellet remains, and resuspend in 1 ml (S) / 2 ml (L) of pre-warmed Type 1 medium.
7. Add 100 µl of collagenase II (20 mg/ml stock, final concentration = 1 mg/ml) and 4 µl of ROCK inhibitor (5 mM stock, final concentration = 10 µM).
8. (S) Wrap the 1.5-ml tube with parafilm and place in the bench-top thermoshaker set to 37ºC and 350 rpm. (L) Wrap the 15-ml tube with parafilm and place at a slight angle (~15˚) in an orbital shaker incubator set to 37ºC and 140 rpm.
9. Digest the tissue in increments of 15 minutes until clusters of 5–10 cells are observed. Pipette up and down 5–10 times every 15 minutes with a P1000 pipette pre-wetted with D-BSA to aid digestion.
   - *For most samples, one 15-minute incubation is sufficient.*
   - *If there is a very large amount of tissue, consider doubling the concentrations of collagenase II and ROCK inhibitor and digesting for longer than 15 minutes.*
10. After incubation, add 200 µl of FBS directly to the tube to stop the digestion. Homogenize the digested tissue by pipetting it up and down vigorously with a P1000 pipette pre-wetted with D-BSA.

11. *(Optional*) For samples with a lot of debris post-digestion, pre-wet a 100-µm strainer (VWR 76327-102) by pipetting 5 ml of D-BSA through the strainer into a new 50-ml conical tube. Swirl the tube to wet the sides of the tube and discard the D-BSA. Then, strain the digested tissue over the 100-µm strainer into the 50-ml tube. Transfer the unfiltered tissue pieces into a second 50-ml tube (pre-wetted with D-BSA) by reversing the filter and flushing with 5 ml of D-BSA. Pipette the digested tissue up and down vigorously with a 5-ml sterile serological pipette pre-wetted with D-BSA. Repeat the straining process two more times, using the same filter to avoid wasting tissue. Centrifuge the flow-through at 300 rcf (S) / 450 rcf (L) for 5 minutes at RT (S) / 8ºC (L). Aspirate the supernatant, leaving the last 800 µl.
12. (*Optional, but recommended*) If the pellet is reddish, add 1 ml of red blood cell lysis buffer with a P1000 pipette, resuspend the pellet by pipetting up and down several times and incubate for 2 minutes at room temperature.
    - *1 ml of red blood cell lysis buffer is enough for pellets <100 µl in size.*
13. Add 0.5 ml (S) / 2 ml (L) of D-BSA and pipette up and down several times. Centrifuge the mixture at 300 rcf (S) / 450 rcf (L) for 5 minutes at RT (S) / 8ºC (L). Aspirate all of the supernatant carefully, so that only the sedimented tissue pellet remains.
14. Resuspend the pellet in an appropriate amount of Matrigel.
    - *As a guideline, 200 µl is sufficient for a pellet of ~50 µl. Samples typically yield enough cells for 30 µl of matrigel.*
    - *The amount of Matrigel to add scales linearly with the pellet volume.*
15. Plate the cell-Matrigel suspension in 5 µl drops, one per well of a 96-well plate or in a column of a 96-stripwell (Corning #9102). Turn the plate upside down and leave in the biosafety cabinet for 5 minutes. Transfer the plate, upside down, into a 37ºC incubator and leave to solidify for 30 minutes.
16. Remove plate from incubator, flip right-side-up, and add 100 µl of pre-warmed Type 2 medium to each well. Transfer to an incubator at 37ºC and 5% CO_2.

Media and buffer recipes (reagent stock concentrations indicated in parentheses)

- adDMEM/F12+++ (for 500 ml total volume, store at 4ºC for up to 6 months):
  500 ml adDMEM/F12
  5 ml penicillin-streptomycin
  5 ml Glutamax
  5 ml HEPES
- D-BSA (for 500 ml total volume, store at 4ºC for up to 4 weeks):
  500 ml DMEM GlutaMAX
  5 ml penicillin-streptomycin (1% vol/vol)
  5 ml 10% BSA (fatty acid free, wt/vol in DPBS) solution
- Type 1 Medium (for 20 ml total volume, store at 4ºC for up to 1 week):
  15.26 ml adDMEM/F12+++
  2 ml R-spondin conditioned medium
  2 ml Noggin conditioned medium
  400 µl B27 (50x)
  200 µl nicotinamide (1 M in DPBS)
  500 µl N-acetyl-L-cysteine (500 mM in water)
  40 µl Primocin (50 mg/ml)
  20 µl Y-27632 (5 mM in DMSO)
  10 µl Heregulin β1 (10 µM in DPBS-B) (DPBS-B = 0.1% wt/vol BSA (modified fraction V) in DPBS)
  10 µl human FGF-7 (10 µg/ml in DPBS-B)
  10 µl human FGF-10 (40 µg/ml in DPBS-B)
  2 µl A83-01 (5 mM in DMSO)
  1 µl human EGF (0.1 mg/ml in DPBS-B)
  0.68 µl SB202190 (30 mM in DMSO)
- Type 2 Medium (for 20 ml total volume, store at 4ºC for up to 1 week):
  11.22 ml adDMEM/F12+++
  4 ml Wnt conditioned medium
  2 ml R-spondin conditioned medium
  2 ml Noggin conditioned medium
  400 µl B27 (50x)
  200 µl nicotinamide (1 M in DPBS)
  500 µl N-acetyl-L-cysteine (500 mM in water)
  10 µl hydrocortisone (1 mg/ml in ethanol)
  20 µl β-estradiol (100 µM in ethanol)
  20 µl forskolin (10 mM in DMSO)
  40 µl Primocin (50 mg/ml)
  20 µl Y-27632 (5 mM in DMSO)
  10 µl Heregulin β1 (10 µM in DPBS-B)
  10 µl human FGF-10 (40 µg/ml in DPBS-B)
  2 µl A83-01 (5 mM in DMSO)
  1 µl human EGF (0.1 mg/ml in DPBS-B)

Adapted from: Dekkers JF, et al. Long-term culture, genetic manipulation and xenotransplantation of human normal and breast cancer organoids. Nat Protoc. 2021;16(4):1936-65.