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Title: Creating Matched In vivo/In vitro Patient-Derived Model Pairs of PDX and PDX-Derived Organoids for Cancer Pharmacology Research

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Current Protocol Length

Total Number of Steps: 17

Introduction

NOTE to VO: Please record the introduction and conclusion statements.

1. Introductory Statements

1.1. This protocol can be used to establish a matched library of paired patient-derived xenografts, or PDX, and corresponding patient-derived organoid lines.

1.1.1. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 2:09 – 2:12.

1.2. The advantage of this method is that it can be used to create organoids using PDX for in vitro screening, resulting in matched pairs of in vivo-in vitro models.

1.1.2. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 6:40 – 6:45.

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Crown Bioscience.

Protocol

2. Preparation for PDX-Derived Organoid Culture

2.1. Begin by harvesting the tumors and processing the tissue as described in the text manuscript. After tissue digestion, pipette the tissue up and down with a 5-milliliter sterile plastic pipette, then add 20 milliliters of AD+++ to the homogenate and filter it with a 100-micrometer cell strainer **[1]**.

2.1.1. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 2:15 – 3:30. *Video Editor: Please use the best-looking clips of talent pipetting the contents of the tube, adding AD+++, and running the tube contents through the strainer.*

2.2. Wash the filtrate twice with AD+++ **[1]**, then transfer it to a plastic tube and centrifuge it at 450 *x g* for 5 minutes. Resuspend the cells in BME and keep them on ice **[2]**.

2.2.1. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 0:10 – 0:25.

2.2.2. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 1:05 – 1:12.

2.3. To prepare the organoid culture, transfer 200 microliters of the cell suspension to each well of a 6-well plate **[1]** and incubate the plate at 37 degrees Celsius for 30 minutes **[2]**.

2.3.1. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 6:12 – 6:55.

2.3.2. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 6:58 – 7:10.

2.4. Add 2 milliliters of organoid media to each well **[1]** and image the representative drops under a microscope **[2]**. Maintain the organoid cultures at 37 degrees Celsius and 5% carbon dioxide with medium changes every 3 to 4 days and passage at a 1 to 2 ratio every 7 days **[3]**.

2.4.1. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 1:20 – 1:45.

2.4.2. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 1:50 – 2:00.

2.4.3. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 2:02 – 2:08.

3. IC_{50} assay

3.1. After dissociating the organoids, run them through a 70-micrometer filter into a 50-milliliter plastic tube **[1]**.

3.1.1. LAB MEDIA: IC50 step1 & PDX Organoid step1_CBSD.mp4. 0:00 – 2:40.

3.2. Count the organoids under a microscope **[1]** and resuspend them in BME on ice for a final concentration of 5% **[2]**.

3.2.1. LAB MEDIA: PDX Organoid step5-6 & IC50 step1-3_CBSD.mp4. 6:40 – end.

3.2.2. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 5:28 – 5:40.

3.3. Add 50 microliters of the organoid suspension into each 384-well plate with a liquid dispenser for a seeding density of 200 CR2110 PDXOs per well **[1]**.

3.3.1. LAB MEDIA: IC50 step4_CBSD.mp4.

3.4. Use the digital dispenser to add SN-38 to each well in serial dilution **[1]**. Create a plate map using the digital dispenser software tool **[2]**.

3.4.1. LAB MEDIA: IC50_step5-7_CBSD.mp4. 0:00 – 0:16.

3.4.2. LAB MEDIA: IC50_step5-7_CBSD.mp4. 0:17 – 0:19.

3.5. The treatments should include a negative control of vehicle with 100% viability and positive control of 5 micromolar starurosporine with 0% viability **[1]**.

3.5.1. LAB MEDIA: IC50_step5-7_CBSD.mp4. 0:19 – end.

3.6. When finished, place the drug-treated 384-well plates back into the 37-degree Celsius incubator **[1]**.

3.6.1. LAB MEDIA: PDX Organoid step2-4_CBSD.mov. 7:05 – 7:07.

Results

4. Results: Morphology, Transcriptome Expression, and Pharmacological Properties of PDXO-CR2110

4.1. Under light microscopy, PDXO-CR2110 shows typical cystic morphology, demonstrating the similarity between PDX-derived organoids and patient-derived organoids under the same culture conditions **[1]**.

4.1.1. LAB MEDIA: Figure 1 A.

4.2. Histopathological examination by H and E staining reveals that the tissue structures and cell types of PDXO-CR2110 are similar to the original PDX-CR2110 **[1]**.

4.2.1. LAB MEDIA: Figure 1 B and C. *Video Editor: Label B "***PDXO-CR2110***" and C "***Original PDX-CR2110***".*

4.3. Genomic profile comparisons of PDX and PDXO demonstrate a 94.92% correlation of transcriptome expression and a 97.67% concordance of DNA mutations, suggesting an overall genomic similarity between this pair of models **[1]**.

4.3.1. LAB MEDIA: Figure 2.

4.4. Drug sensitivity assays were performed on PDXO-CR2110 in 384-well plates. PDXO-CR2110 was sensitive to irinotecan and resistant to cisplatin **[1]**, consistent with PDX treatment results **[2]**.

4.4.1. LAB MEDIA: Figure 3 A.

4.4.2. LAB MEDIA: Figure 3 B.

Conclusion

5. Conclusion Interview Statements

5.1. The matched pair of in vitro and in vivo models can complement each other for in vitro screening and validation in vivo, improving the success rate of drug discovery and potentially reducing attrition rates in clinical development.

5.1.1. LAB MEDIA: PDX Organoid step5-6 & IC50 step1-3_CBSD.mp4. 0:00 – 0:20.