Patient-derived organoid drug responses corroborate known target–drug interactions for selected anticancer agents

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Abstract:
Patient-derived organoids (PDOrgs) are heterogeneous three-dimensional cellular clusters originating from human cancer tissues. PDOrgs have been used to model human cancers in vitro and serve as an ideal system to assess drug responses. This study assessed drug sensitivities of PDOrg models in the National Cancer Institute’s (NCI) Automated Primary Screening System (APSS) conducted at the National Cancer Institute Frederick (NCIF Frederick). PDOrgs were derived from patient-derived xenografts (PDXs) of colorectal and non-small cell lung adenocarcinomas. The PDOrgs were treated with known drugs or their vehicle controls, and growth rate inhibition was measured at 96 and 144 hr post-drug addition. Correlations between ABCB1 mRNA expression and ABCB1 substrate drug responses in PDOrgs were investigated. We found that PDOrgs expressing the KRAS G12C variant are, overall, more sensitive to covalent KRAS G12C inhibitors than to other ABCB1 substrates. PDOrgs expressing the KRAS G12C variant are, overall, more sensitive to covalent KRAS G12C inhibitors than to other ABCB1 substrates. This study demonstrates the ability of organoids to serve as useful models for evaluating therapeutic responses to anticancer agents, including identifying known target–drug associations.

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Organoid Models, platining densities and status/expression of genes of interest

Organoid models harboring KRAS G12C are, overall, more sensitive to RAS-targeting agents

Good correlation between ABCB1 mRNA expression and doxorubicin responses in PDOrgs

Table: ABCB1 mRNA expression and doxorubicin responses in PDOrgs

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ABCB1 EXPRESSION (%)</th>
<th>DOXORUBICIN RESPONSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1 (high)</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>ABCB1 (low)</td>
<td>No</td>
<td>Low</td>
</tr>
</tbody>
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Methods:
Organoid Growth and Preparation:
Organoids were grown in 100% BME medium either to fixed plates or in 100% BME media. Cell cycle inhibitors were added to plates at 96 hr to allow the cells to exit G0/G1. PDOrgs were seeded into 384-well microplates at a density of 5,000 cells per well.

Organoid Inoculation onto 384-Well Plates:
The organoid suspension was inoculated into black, 384, clear-bottom polypropylene plates designed for use in the Echo Acoustic Liquid Handler (EALH) (Beckman Coulter, Inc., Brea, CA). The EALH was used to deliver the organoid inoculum into each well of the 384-well plate, with 30 μl of organoid suspension per well. The plates were then placed on a BioShake XP (QInstruments, Germany) for 5 min at 1,200 rpm to allow the organoids to settle onto the plate.

Drug and Vehicle Addition:
Approximately 20 μl of 10X drug stock vehicle and drug solutions were added to the plates using the Echo Acoustic Liquid Handler (EALH) using the Echo Acoustic Droplet Robotics (ADRs) automated dispensing system (Lynx Tec Robotics, Costa Mesa, CA), which dispenses up to 1,000 μl of liquid to the rows and columns of the plate and sequentially disperses it on to the cells. The plate was then placed on the BioShake XP for 5 min at 1,200 rpm to allow the organoids to settle onto the plate. The plates were then incubated at 37°C and 5% CO2 for 4 hr.

Measurement of Organoid Viability:
Organoids were measured at two different time points, 96 and 144 hr post-drug addition. The viability of organoids was determined using the Echo Acoustic Liquid Handler (EALH) using the Echo Acoustic Droplet Robotics (ADRs) automated dispensing system (Lynx Tec Robotics, Costa Mesa, CA), which was used to deliver the organoid inoculum into each well of the 384-well plate, with 30 μl of organoid suspension per well. The plates were then placed on the BioShake XP for 5 min at 1,200 rpm to allow the organoids to settle onto the plate. The plates were then incubated at 37°C and 5% CO2 for 4 hr.

Data Analysis:
Event was used to gate the viable organoids. Viable organoids were defined as those with two or more nuclei, as determined by the Echo Acoustic Liquid Handler (EALH) using the Echo Acoustic Droplet Robotics (ADRs) automated dispensing system (Lynx Tec Robotics, Costa Mesa, CA). The number of viable organoids was determined at each time point, and the growth rate inhibition was calculated as the ratio of the growth rates in the vehicle control and drug-treated wells. The viability of organoids was measured at two different time points, 96 and 144 hr post-drug addition. The viability of organoids was determined using the Echo Acoustic Liquid Handler (EALH) using the Echo Acoustic Droplet Robotics (ADRs) automated dispensing system (Lynx Tec Robotics, Costa Mesa, CA), which was used to deliver the organoid inoculum into each well of the 384-well plate, with 30 μl of organoid suspension per well. The plates were then placed on the BioShake XP for 5 min at 1,200 rpm to allow the organoids to settle onto the plate. The plates were then incubated at 37°C and 5% CO2 for 4 hr.

Summary:
• The assay was able to identify sensitivities to targeted agents (KRAS G12C, BRAF V600E, and ERBB2/HER2) and other ABCB1 substrates (AZD1775, trametinib, and SN-38) in PDOrg models.
• PDOrgs expressing the KRAS G12C variant are, overall, more sensitive to covalent KRAS G12C inhibitors than to other ABCB1 substrates.
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