Utilizing the zebrafish xenograft model to evaluate anticancer drug efficacy
Overcoming therapeutic resistance with Bcl-2 functional converters.
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**Background**

- The zebrafish xenograft model is a powerful tool for assessing tumorigenic potential and drug efficacy.
- Model provides an in-vivo, high-throughput, low-cost platform for drug-screening and toxicity assessment.
- Provides a more robust, and representative model of cancer-drug response than cell culture.
- We leverage this model for evaluating anti-cancer action of Bcl-2 functional converter (BFC) compounds.
- Bcl-2 is upregulated in therapy-resistant cancers as a mechanism of acquired resistance.
- BFCs convert Bcl-2 from a pro-survival protein to a pro-apoptotic conformation, exposing the BH3-death domain.
- Palbociclib (Pd) is a recently approved (2015) clinical drug currently used for ER-positive/HER2-negative breast cancer treatment. Pd is a CDK4/6 inhibitor, preventing cell-cycle progression.
- Pd is initially effective, but acquired therapy-resistance still observed in the clinic.
- Urgent need still exists for targeting resistant cancers.

**Methods**

Zebrafish Xenograft Experiments: Fish were acquired from Sinhuber Aquatic Research Laboratory and maintained in (.0003%) phenylthiourea in E3 media following 24 hpf to prevent melanization. Pd-resistant MCF7 cells were previously generated by continuous culture in drug for 6-8 weeks with an increasing concentration regimen (.001-1 μM). The day of injection, MCF7 cells were stained with fluorescent CM-DiI and injected into the yolk sac of 48 hpf fish by air-driven micro-pressure injector via a borosilicate glass needle. Cell suspension was 1 x 10⁷ cells/ml, and approximately 200 cells were used per fish.

Fish were imaged 1 day and 4 days post-injection. For imaging, fish were anesthetized by 0.2 mg/mL Tricaine and embedded in 0.8% low melting point agarose in a 96-well plate. Imaging was performed with the High-content microscope, with 2 stacks captured on a 10X objective.

Cancer growth was analyzed using Fiji software, by making a maximum projection image of the z-stack and applying a median filter. Area was calculated by creating a binary mask from thresholds with the Otsu algorithm and calculating total area of resulting segmented objects.

**Results**

**A) Generation of a therapy-resistant breast cancer line – MCF7 cells become insensitive to Pd**

**B) Pd-Resistant cells are more sensitive to B18 treatment than parental cells.**

**C) B18 reduces tumor burden in Pd-resistant xenografts**

**D) B18 suppresses tumor growth**

Conclusions and Future Directions

- Bcl-2 represents a logical molecular target for overcoming acquired resistance to therapy and is upregulated in numerous cancers, including ER-positive breast cancer.
- Bcl-2 functional conversion by B18 is capable of suppressing Pd-resistant MCF7 cells in the zebrafish xenograft model, and showed no signs of overt toxicity to fish.
- BFCs have further shown efficacy in breast cancer xenografts resistant to other therapeutics such as paclitaxel (data not shown here).
- Future directions include:
  - Evaluating B18 activity in xenografts with parental MCF7 to demonstrate differential sensitivity in this model.
  - Demonstrating Bcl-2 functional conversion as a mechanism of suppression by staining for BH3 domain exposure in sectioned zebrafish.