Patient derived xenografts in zebrafish embryos (Zevatars) demonstrate differential drug responses to pancreatic cancer chemotherapeutics.

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Background

Cancer is currently the second leading cause of death in the world. Given that cancer is a highly individualized disease, it is very difficult to accurately predict the best chemotherapeutic treatment for individual patients. Ex vivo models such as mouse PDX and organoids are being developed to determine patient tumor responses before clinical treatment. Although promising, these models pose significant disadvantages including long growth times that introduce tumor genetic changes and high costs. To overcome these issues, we have developed a zebrafish xenograft assay (Zevatars) to evaluate the efficacy of chemotherapies on patient biopsies in a model that recapitulates the original tumor as closely as possible. Our preliminary focus is pancreatic cancer, the most lethal solid malignancy. Samples of primary pancreatic ductal adenocarcinoma (PDAC) or liver metastases from patient tumor biopsies are cut into 50µm pieces, fluorescently labeled, and implanted into 2-day-post-fertilization (dpf) embryos. The implanted embryos are treated with maximum tolerated doses of gemcitabine+abraxane and folfirinox (current standard of care drugs) and are imaged immediately after treatment and re-imaged 2-5 days post-implantation to determine tumor response. Chemotherapy tumor response is assessed by change in tumor area and presence of metastasis using ImageJ. Currently, we have implanted over 1000 embryos with patient biopsy samples and have demonstrated that our assay can determine differential drug responses and is effective in doing so with either fresh or cryopreserved tissue. The Zevatar assay is ideal for large-scale chemotherapy screening and is a rapid, cost-effective method to positively impact prescription of effective chemotherapies for individual cancer patients.

Overview

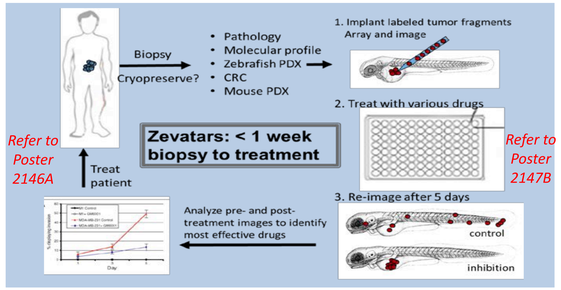


Figure 1. Summary of implantation process.

Materials and Methods

Tumor preparation, Labeling and Transplantation

- Patient tumor biopsies are collected and cut into 1mm^3 pieces with hypodermic needles for implantation or cryopreservation.
- Pieces for cryopreservation are placed in freezing media (10% DMSO, 90% Fetal Bovine Serum) and stored at -80ºC.
- The fragment for implantation is then further cut into 50-80µm^3 pieces and fluorescently labelled with CM-dil for 30 min.
- The labelled pieces are then implanted into the yolk of 2dpf embryos using tungsten needles.

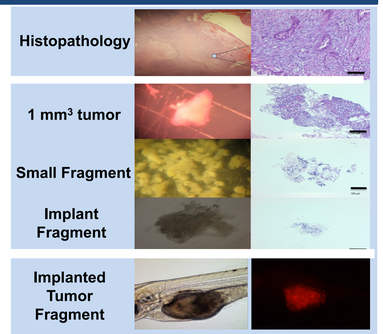


Figure 2. Fragmentation, labeling and implanting PDAC tumor tissue. The histopathology of the tumor is shown along with the histology as the tumor is cut into small fragments for labeling and even smaller fragments for implantation.

Results

Fresh or cryopreserved tumors are viable in Zevatars

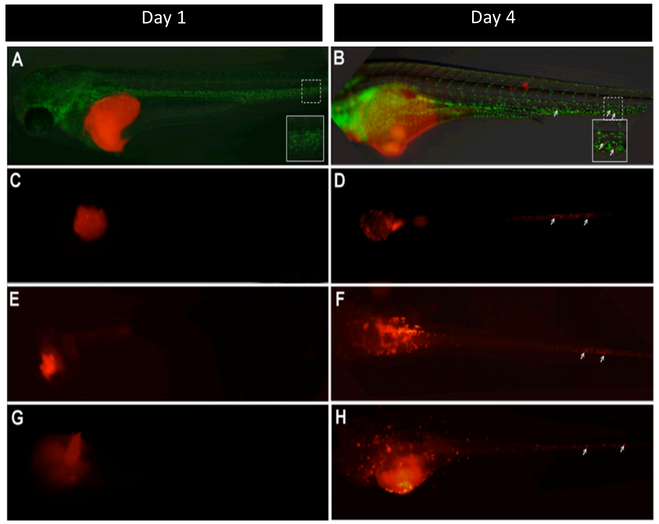
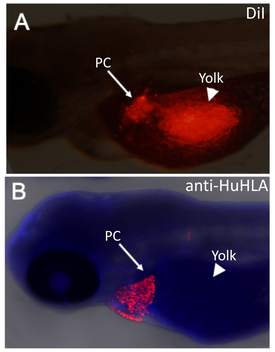
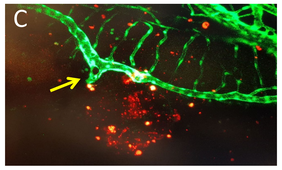


Figure 3. Visualization of tumor growth and metastasis in Zevatars. Fresh (A-D) or cryopreserved (E-H) tumor fragments were implanted into 2 dpf embryos and imaged 1 day post implantation (A,C,E,G). The embryos were imaged again 4 days post implantation (B,D,F,H). (A,B): Tumor piece was implanted in *fli:nGFP* transgenic embryos (green vasculature). Green vasculature and red tumor images were merged to illustrate the location of migrating tumor cells. White arrows indicating metastasizing cells. Data suggests that cryopreserved tumors can be used to generate Zevatars.

Vascularization, Metastases, and Confirmation of Human Cells in Fresh and Cryopreserved Tumor Tissue





Biopsy ID	Zevatar Fresh (n)	Mets	Zevatar Cryo (n)	Mets
1A1340	9	ND	32	8
1A1390	47	25	13	0
1A1395	50	19	TBD	TBD
1A1399	59	45	TBD	TBD
1A1400	24	18	TBD	TBD
1A1408	ND	ND	8	0
1A1429	41	33	26	18
1A1442	53	36	TBD	TBD

Figure 4. A,B, Immunofluorescence staining. (A) CM-dil labeled tumor cells. (B) Cells in the paracardial cavity (PC) stained with anti-Human HLA antibody, while cells in the yolk remain unstained. (C) Vascular bud growing out of the hepatic vein (arrow) entering the tumor xenograft (red). Table 1: metastatic tumor cells are found in embryo xenografts both fresh and cryopreserved tissue.

Differential Drug Response in Zevatars

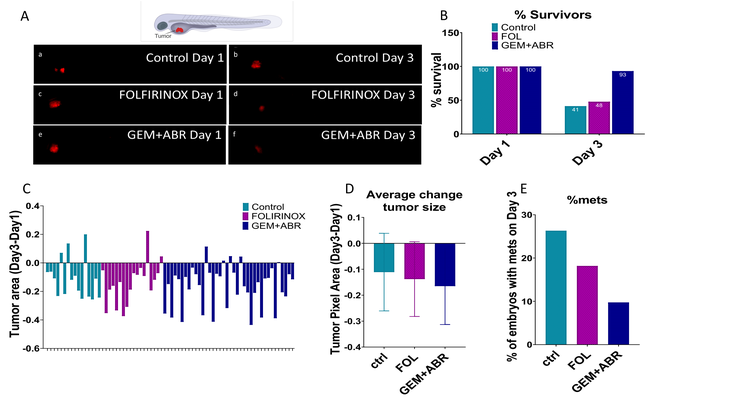


Figure 5. Differential drug response of liver metastasis biopsy (primary pancreatic cancer): **A**, Images of implanted tumor, (a,c,e) day 1 and (b,d,f) 3 days post treatment. (a,b) control; (c,d) folfirinox (FU, folinic acid, irinotecan, oxaliplatin); (e,f) gemcitabine (GEM)+Abraxane (ABR). **B**, Percentage of survivors after 3 days of treatment. **C**, Change in object voxels of the tumor between Day 1 and Day 3 in each embryo in each group. **D**, Average change in object voxels between treatment groups n(ctrl)=46, n(FOL)=46, n(GEM)=44. **E**, Percentage of implanted fish that had metastasis in tail after drug treatment for 3 days. Error bars represent standard deviation of replicates in each treatment group. Statistical significance was calculated using student T-test.

Metastasis Quantification

Table 2: Primary pancreatic tumors

Biopsy ID	Control		100uM Gemcitabine	
	Total	Mets (%)	Total	Mets (%)
1A1395	24	9 (38)	26	10 (38)
1A1399	30	26 (87)	29	19 (66)
1A1400	12	10 (83)	12	8 (67)
1A1442	25	20 (80)	28	16 (57)

Table 3: Liver mets (primary pancreatic & colorectal cancer)

Biopsy ID	Control		Drug Treated	
	Total	Mets (%)	Total	Mets
1-10292018-A	4	0 (0%)	GEM:7	0 (0%)
1-11012018-A	1	0 (0%)	GEM:6	1 (17%)
1-12112018-A	7	0 (0%)	GEM:22 FOL:16	5 (23%) 3 (19%)
1-12282018-A	8	1 (13%)	GEM:9	0 (0%)
1-01102019-A	4	4 (100%)	GEM:5	5 (100%)
1-01222019-A	8	3 (38%)	FOL:11	6 (55%)
1-01242019-A	7	3 (43%)	GEM:6 FOL:3	0 (0%) 1 (33%)
1-02182019-A	13	0 (0%)	GEM:13	0 (0%)
1-02252019-A	6	1 (17%)	GEM:8 FOL:3	0 (0%) 0 (0%)
1-03132019-A	23	4 (17%)	GEM+A: 46 FOL: 30	4 (9%) 0 (0%)

• Conclusions and - Future Directions

- Zevatars are a diagnostic tool to assess patient tumor responses to drugs *ex vivo*
- Patient tumor response can be assessed in **less than 1 week**
- Rapid and cost effective
- Zevatars can be used for personalized medicine

- Assess versatility of the Zevatar assay using other tumor types
- Use immunofluorescence for markers of tumor proliferation and apoptosis
- Develop high throughput imaging analysis methods
- Utilize Zevatars to validate the predictive value of tumor molecular profiling

Poster template courtesy Faculty & Curriculum Support (FACS), Georgetown University School of Medicine