Zebrafish Orthotopic Brain Cancer Model for Drug Screening

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Abstract

Brain cancer accounts for approximately 10 - 30% of adult cancers and many forms of cancer can metastasize to the brain, leading to death. Although immunodeficient mice and rats have played an important role as hosts for brain cancer xenografts, a simple and cost-effective orthotopic animal brain cancer model will improve efficiency of drug screening. In this study, approximately 150 human neuroblastoma BE (2)–C cells were microinjected into 2 day post fertilization (dpf) zebrafish brains. After orthotopic xenotransplant, brain cancer cells proliferated and formed masses. Zebrafish survived through five days, providing an adequate time window for drug screening. Next, we developed a quantitative microplate-based whole zebrafish chemiluminescence ELISA. This assay format relies on anti-human Survivin monoclonal antibody specific for cancer cells. In pilot drug tests, xenotransplant zebrafish were treated with anti-cancer drugs: Doxorubicin, Paclitaxol and Curcumin at varying concentrations from 1 day post xenotransplant (dpi) to 4 dpi (3 dpf to 6 dpf). At the end of treatment, the orthotopic neuroblastoma cells were quantitated using the anti-human Survivin antibody-based ELISA. All 3 drugs significantly decreased chemiluminescence signal from drug-treated xenotransplant zebrafish, compared with vehicle-treated controls. Additional experiments to further validate our zebrafish orthotopic brain cancer model for drug screening are ongoing. These preliminary data suggest that the microplate-based whole zebrafish chemiluminescence ELISA is specific, sensitive, and comparatively high throughput. Furthermore, drug results in zebrafish were predictive of drug results in mammals.

Introduction

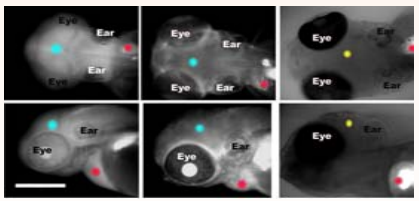
Brain Cancer

- Brain cancer is the leading cause of cancer deaths in children and is also a leading cause of death in patients over the age of 65
- Currently, in the US, 360,000 people are currently living with brain tumor diagnosis
- 190,000 people were diagnosed with a primary or secondary brain cancer in 2008
- Approximately 17,000 Americans die of primary brain cancer every year
- Brain cancers are not curable and a signficant obstacle to treatment is BBB permeability

Zebrafish as a Xenotransplant Model for Drug Screening

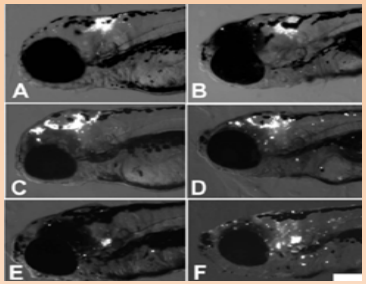
- Hundreds of cells per animal are sufficient for transplantation
- Small amount of drug (µg/animal) is required per test
- Drugs can be added directly to the fish water
- Statistically significant number of animals can be used per experiment
- Approximately 200 animals can be injected per hour
- BBB is present in all vertebrates, including zebrafish
- Whole animal ELISA is rapid, reproducible and cost effective

Zebrafish Exhibit a Functioning BBB



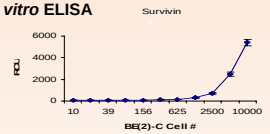
Fluorescein sodium salt was injected into the circulation of each animal (red dots), and detected in the brain of 2 and 3 dpf zebrafish (blue dots). In contrast, no dye was detected in the brain of 4 dpf zebrafish (yellow dots). These data indicate that BBB has formed in 4 dpf zebrafish. Scale bar = 200 µm.

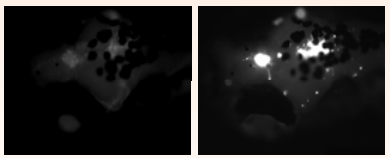
Xenotransplant Human Brain Cancer Cells Proliferate and Migrate in Zebrafish Brain



CM-DiI-labeled cancer cells were injected into the hindbrain ventricle of 2 dpf zebrafish. A,B. 150 neuroblastoma BE(2)C cells shown 1dpx (A) and 5 dpx (B) in the same zebrafish. C,D, 150 medulloblastoma D341 cells shown 1 dpx (C) and 5 dpx (D) in the same zebrafish. E,F, 100 malignant glioblastoma M059J cells shown 2 dpx (E) 1 dpx and 5 dpx (F) in the same zebrafish. Since the total fluorescent signal in CM-DiI dye remains constant, proliferation of cancer cells that do not migrate from the injection site cannot be reliably assessed by this method. Insignificant migration was observed for BE(2)C (A, B) and D341 cells (C,D). In contrast, glioblastoma M059J cells were highly migratory (arrows) and number of xenotransplanted cells appeared to increase. Scale bar (F) represents 200 mm.

Human Neuroblastoma BE(2)C Cell Number-Dependant Increase in Chemiluminescence Signal in Anti-human Survivin Antibody-based *in vitro* ELISA

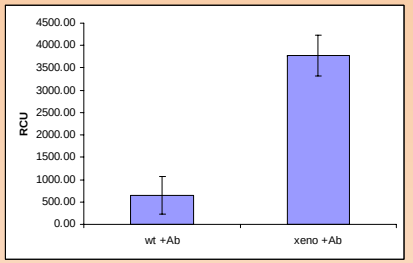




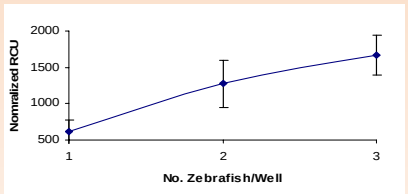
Survivin Staining CM-DiI

Whole mount immunostaining of human neuroblastoma BE(2)C cells xenotransplanted into zebrafish. CM-DiI-labeled cells were visualized under rhodamine channel (right) and Survivin antibody-stained zebrafish were visualized under FITC channel (left).

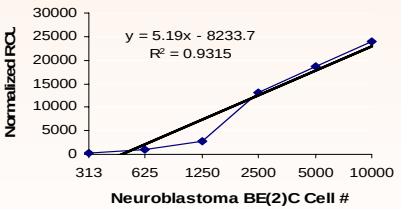
5 dpf zebrafish in 96-well microplate, one zebrafish/well



Whole xenotransplant ELISA using anti-hSurvin antibody. 2 dpf zebrafish were xenotransplanted with 150 neuroblastoma BE(2)C cells and fixed for ELISA processing 4 days later. After subtracting corresponding background signal, data were expressed as Mean ± SE from (n =10 wells, 3 zebrafish/well). Y axis represents relative chemiluminescence units (RCU). Very significant difference ($p < 0.01$) was observed between xenotransplant and wild type uninjected zebrafish, indicating signal from cancer cells can be accurately quantitated by ELISA.



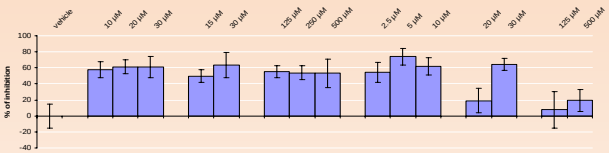
Linear Relationship between increase in chemiluminescence signal and number of xenotransplant zebrafish per well. A linear relationship was observed between signal increase and zebrafish number per well using anti-hSurvin monoclonal antibody. Data were presented as mean ± SE from 10 measurements. Normalized RCU: normalized relative chemiluminescence units.



Quantitating human neuroblastoma BE(2)C cells in xenotransplant zebrafish. Based on the *in vitro* standard curve, there were approximately 650 human neuroblastoma BE(2)C cells in a single xenotransplant zebrafish 4 days post transplantation. Data was expressed as Mean ± SE from 10 measurements

LC_{10} and Selected Drug Concentrations

Drugs	LC_{10}	Test Concentrations
Doxorubicin	40 µM	40, 20, 10 µM
Paclitaxel	(highest solubility 30 µM)*	30, 15, 7.5 µM
Carboplatin	(highest solubility 500 µM)*	500, 250, 125 µM
Curcumin ASC-J9	(highest solubility 20 µM)*	20, 10, 5 µM
Carmustine (BCNU)	87 µM	87, 43.5, 21.75 µM
Temozolomide	960 µM	960, 480, 240 µM
Tamoxifen	7.7 µM	7.7, 3.85, 1.93 µM
Lomustine	13.2 µM	13.2, 6.6, 3.3 µM



Effects of anti-cancer drugs on human neuroblastoma BE(2)C cells xenotransplanted into zebrafish brain, measured by ELISA. Cancer cell inhibition was expressed as % of vehicle control (C). Doxorubicin, Paclitaxel, Carboplatin, Curcumin ASC-J9 and Carmustine inhibited proliferation and/or killed cancer cells ($p < 0.05$ or $p < 0.01$, compared with vehicle control, except 15 µM Paclitaxel and 20 µM Carmustine. Temozolomide did not exhibit any inhibitory effect.

Comparison of Brain Cancer Drug Effects in Zebrafish and Mammals

Compounds	Pharmaceutical Function in Mammals	Cancer Inhibition in Zebrafish	Cancer Inhibition* in Mammals	Correct Prediction
Doxorubicin	Inhibits DNA synthesis and induces apoptosis	Inhibition	Inhibition	Yes
Paclitaxel	Mitotic inhibitor	Inhibition	Inhibition	Yes
Carboplatin	Induces apoptosis in cancer cells	Inhibition	Inhibition	Yes
Curcumin ASC-J9	Inhibit the growth of cancer cells	Inhibition	Inhibition	Yes
Carmustine	DNA alkylating agent	Inhibition	Inhibition	Yes
Temozolomide	DNA methylating agent	No inhibition	Inhibition	No

*Overall prediction rate = 83.3% (5/6)

Conclusions

Zebrafish brain cancer xenotransplant model combined with chemiluminescence ELISA shows promise as a rapid, reproducible, predictive animal model for drug screening.

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