

SUMMARY

In one aspect of the present disclosure, there is provided a method to establish tumors from circulating tumor cells (CTCs) obtained from biopsies for analysis of said CTCs comprising:

- a. Isolating one or more circulating tumor cells;
- b. Labeling the circulating tumor cells with a cell tracking dye;
- c. Injecting the circulating tumor cells into a 24 to 48 hours post fertilization (hpf) zebrafish embryo; and
- d. Incubating the embryo for 24 hours or more.

In a further aspect of the present disclosure, there is provided a method for testing a drug for its effect on circulating tumor cells, comprising:

- a. microinjecting one or more circulating tumor cells into a 24 to 72 hour post-fertilization zebrafish embryo;
- b. allowing the circulating tumor cells to grow within the zebrafish embryo for a predetermined time;
- c. incubating or microinjecting the zebrafish embryo with a drug; and

- d. monitoring the effect of the drug on the circulating tumor cells by measuring the circulating tumor cells in the embryo after the pre-determined time.

AIMS OF THE PRESENT DISCLOSURE

The aims of the present disclosure were to overcome the technical problems of profiling and characterizing primary tumor cells and CTC in an animal model, and to provide a system that would enable efficient manipulation and injection of zebrafish embryos.

The applicant has discovered that these technical problems are solved by way of methods that include creating viable tumors from xeno -transplanted human CTCs into zebrafish, studying the metastatic potential of the injected CTCs, predicting the organ preference of isolated CTCs, and assessing the response to therapeutic agents.

STATEMENTS OF THE PRESENT DISCLOSURE

In one aspect of the invention, a method is provided to develop three-dimensional tumors from primary tumor cells obtained from biopsies by steps comprising:

- (a) Isolating tumor cells
- (b) Labeling tumor cells with a cell tracking dye
- (c) Injecting the cells into a 24 to 48 hours post fertilization (hpf) zebrafish embryo
- (d) Incubating the embryos for 24 hours or more

In one embodiment, the tumor cells are obtained from circulating tumor cells (CTCs).

In another embodiment, the cell tracking dye is a fluorescent dye.

CLAIMS

What is claimed is:

1. A method to establish tumors from circulating tumor cells (CTCs) obtained from biopsies for analysis of said CTCs comprising:
 - a. Isolating one or more circulating tumor cells;
 - b. Labeling the circulating tumor cells with a cell tracking dye;
 - c. Injecting the circulating tumor cells into a 24 to 48 hours post fertilization (hpf) zebrafish embryo; and
 - d. Incubating the embryo for 24 hours or more.
2. The method of claim 1, wherein the cell tracking dye is a fluorescent dye.
3. The method of claim 1, wherein the circulating tumor cells are injected into a 24 to 48 hpf zebrafish embryo yolk.
4. The method of claim 3, further comprising measuring the circulating tumor cells' position after incubating the embryo to determine whether the circulating tumor cells (i) invade the embryo body; or (ii) remain in the yolk sac.
5. The method of claim 4, further comprising using the circulating tumor cells' position to indicate circulating tumor cell metastatic potential, wherein if the circulating tumor cells invade the embryo body, the circulating tumor cells have metastatic potential.
6. The method of claim 4, wherein the circulating tumor cells' position is measured by capturing one or more fluorescence images of the circulating tumor cells under a fluorescent microscope.
7. The method of claim 6, further comprising quantitating the circulating tumor cells in the embryo yolk sac and the embryo body by one or more of:

18. The method of claim 17, further comprising observing the organ-homing pattern change of the injected circulating tumor cells in the absence versus the presence of a drug.

19. A method for testing a drug for its effect on circulating tumor cells, comprising:
 - a. microinjecting one or more circulating tumor cells into a 24 to 72 hour post-fertilization zebrafish embryo;
 - b. allowing the circulating tumor cells to grow within the zebrafish embryo for a predetermined time;
 - c. incubating or microinjecting the zebrafish embryo with a drug; and
 - d. monitoring the effect of the drug on the circulating tumor cells by measuring the circulating tumor cells in the embryo after the pre-determined time.
20. The method of claim 19, further comprising the addition of a pro-angiogenic factor into a water containing the zebrafish embryo either before, during or after the microinjection of the circulating tumor cells.
21. The method of claim 20, wherein the pro-angiogenic factor is angiopoietin.
22. The method of claim 21, further comprising the addition of a pro-angiogenic factor by microinjection into the embryo during the microinjection of the circulating tumor cells.