SHEDDING LIGHT ON THE EFFECTS OF RADIATION THERAPY ON CIRCULATING TUMOR CELLS

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Many common treatments for cancer – including radiation therapy (RT) – have the unfortunate side effect of promoting the spread of cancer to other organs [1-3]. While the 'pro-metastatic' effects of RT have been known for some time, it has garnered renewed attention in recent years in part due to the widespread study of circulating tumor cells (CTCs). In hematogenous metastasis, CTCs detach from the primary tumor and spread via the blood to other organs and tissues of the body. There are three main hypotheses for RT induced metastasis (RTIM) as reviewed in [1]: i) RT causes disruption of the primary tumor and vasculature, which leads to immediate shedding of CTCs, iii) RT induces biomolecular changes in tumor cells, such as epithelial to mesenchymal transition, leading to increased CTC shedding over time as the tumor cells die, and, iii) Systemic effects, such as the elimination of suppressive signaling molecules by the primary tumor resulting in the proliferation of existent but previously dormant micro-metastases [3].

Our team recently developed a new instrument called 'Diffuse *in vivo* Flow Cytometry' (DiFC; *figure 1*) [4]. The main advantage of DiFC is that it samples large circulating blood volumes (hundreds of µL per minute), allowing in vivo detection of very rare CTCs. DiFC uses specially designed fiber-optic probe bundles with built-in filters and lenses for efficient collection of weak fluorescent signals and blocking of tissue autofluorescence. As labeled cells pass through the DiFC field of view, transient fluorescent peaks are detected. A custom signal processing algorithm allowed us to determine the number, direction, speed, and depth of circulating cells, and reject false alarm signals from motion artifacts. For example, we recently showed that DiFC allowed detection of early dissemination of green fluorescent protein (GFP)-labeled multiple myeloma cells in a disseminated xenograft model at CTC burdens below 1 cell per mL, as well as rare CTC clusters (*fig. 1*).

In this presentation, we first discuss the design and prior validation of the DiFC instrument. Second, we discuss our recent work in application of DiFC to the study of RTIM. Specifically, we grew sub-cutaneous Lewis Lung Carcinoma (LLC) tumors in mice, which are known to aggressively metastasize to the lungs via the vasculature. Irradiation of the primary LLC tumors is known to significantly increase pulmonary metastases in this model, by as much as a factor of 5 [3]. We performed local RT of LLC tumors with a Small Animal Radiation Research Platform (SARRP). We measured CTC and CTC cluster numbers with DIFC in response to single or fractionated RT doses, compared to un-irradiated controls. We also monitored metastases in the lungs by BLI imaging, weight, and histology. DiFC revealed that LLC-CTC numbers significantly increased during tumor response in RT mice versus controls, and that these correlated with lung tumor burden at sacrifice. We also discuss future prospects for the use of DiFC in monitoring CTC response to other therapies.

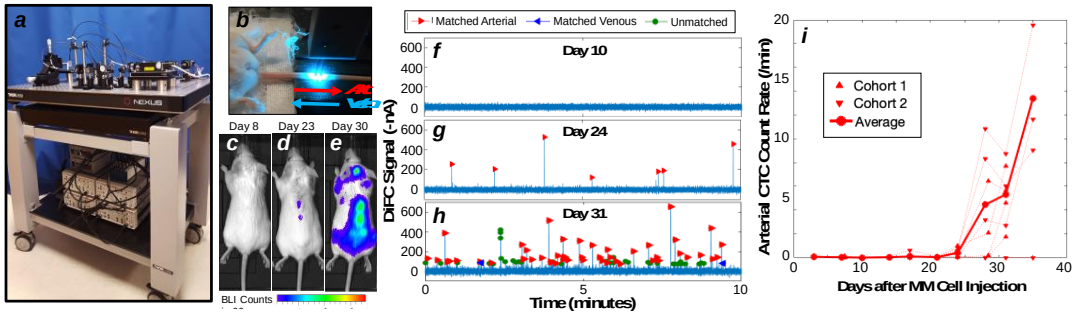

Figure 1. (a) DiFC instrument [4,5] designed to detect fluorescently-labeled CTCs in (b) mouse tail vasculature. We used DiFC to study growth of multiple myeloma in a mouse model, which was also monitored by BLI (c-e). Increasing numbers of CTCs were detected (f-h) in arterial blood (red arrows) as the (i) disease progressed.

References

[1] O.A. Martin et. al., Nat Rev Clin Oncol, 14(1):32-44, 2017; [2] B.J. Blythe et. al., Clin Exp Metastasis, 35(4):223-236, 2018; [3] K. Kamphausen et. al., Cancer Res, 61:2207-2211; [4] X. Tan et. al., Sci Rep, 9(1)3366, 2019; [5] R. Patil et. al. (In review), bioRxiv 516641, 2019.