Evaluation of the effect of B16F10 melanoma cells from 2D and 3D (CSCs/cancer stem cells-enriched) culture on seeding into the murine thymus

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A tumor mass is heterogeneous or consisting of various cell populations including a bulk of fast-cycling cancer cells, and a small fraction of slow-cycling cancer stem cells (CSC-like cells)1. It is proposed that fast and slow-cycling cancer cells can be converted into each other, which is induced by microenvironmental stimuli. CSCs are also theorized to be cancer-initiating cells due to their abilities to terminally differentiate into fast-cycling tumor bulk cells and metastasize to distant sites2. In addition, CSCs are able to resist chemotherapy due to their slow-cycling feature. An example of such metastatic cancer is melanoma3. Dr. Su’s lab has previously found that B16 melanoma cells can harbor in the thymus to become chemo-resistant when injected intravenously (i.v.)4. From this finding, we hypothesize that B16F10 CSCs, formed through in vitro culture, may have a greater ability of either inducing metastatic recurrence or harboring in the thymus, a reservoir for CSCs. We utilized the B16F10 GFP+ mouse melanoma cell line to generate 2D (fast-cycling cancer cells) and 3D (CSC-like cells), via “hanging drop”, cultures. We inoculated C57/BL6 (wt) mice with 2D or 3D (CSCs-like enriched) cells, examined the percentage of GFP+ cells harboring in the thymus, and the cancer lesions in the lungs. (1) We successfully generated 2D and 3D B16F10 GFP+ melanoma cells. Flow cytometric analysis of cells in 3D culture for CSC-associated markers (CD24, CD44, CD133) indicated that the CSC-like population was indeed enriched. (2) Post-injection, analysis of extracted thymuses showed no significant difference in GFP+ cell population between the 2D control vs. 3D experimental groups of mice. However, more cancer lesions were observed in the lungs of the 3D experimental group indicative of increased metastasis. Our preliminary results demonstrate that: (1) we are able to enrich a CSC-like population in B16F10 melanoma cell line using 3D culturing method; (2) CSC-like enriched population does not demonstrate the enhanced capacity to harbor in the thymus, but has an increased tumorigenic ability to form cancer lesions in the lungs, compared to the 2D control group.
References
4. Sizova et al., 2018, in press