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Formation of Stable Small Cell Number Ovarian Cancer

Stem Cell Spheroids for Individualized Therapy

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In recent years, Ovarian Cancer has become the fifth leading cause of cancer deaths among women. The low survival rate of patients with epithelial ovarian cancer can be attributed to its lack of specific symptoms. This late diagnosis gives the cancer time to spread throughout the patient's pelvis and abdomen. An increasing body of evidence has suggested a link between cancer stem cells (CSCs), and tumor growth potential. CSCs are undifferentiated cancer cells which represent a very small percentage of the cells within human tumors (about 0.01%). Therefore, a feasible treatment to limit tumor growth potential would be to target and eliminate CSCs within a tumor. The difficulty in this is that the low concentration and fragility of the CSCs make them difficult to work with when doing experiments. Therefore, the biomedical research community would benefit from a reliable physiological three-dimensional (3-D) model to explain the relationships between the ovarian cancer cells and their supporting cells while still accommodating for the fragile conditions of the CSCs. In the body, ovarian cancer cells grow as spheroids within the peritoneal cavity. This situation is simulated using a 384-well hanging drop platform. We aim to optimize this physiological model to allow long-term culture of small numbers of both ovarian cancer cells and ovarian CSCs. We will also use this 3-D model system screen drug candidates for their ability to treat metastatic ovarian cancers. The results from this project will have an impact on the treatment strategies of ovarian cancers, potentially allowing for individualized therapy. This semester's work was to begin characterizing several patient cell lines grown in the 384-well hanging drop platform. Each cell line was grown alone and in a co-culture with cancer-associated mesenchymal stem cells (CaMSCs), which are the type of CSCs most closely-associated with epithelial ovarian cancer. The cells were kept in culture for between 7 and 14 days, during which, metabolic activity was determined using Alamar Blue and recorded by a plate-reader. Additionally, a micrograph was taken of the cells every two days over the first two week period to visually record growth.

It took a few weeks into the semester to begin work in earnest, due to the lab's recent move up to the [Research Complex], and the time necessary to learn how to use the 384-well hanging drop plates. There was a steep learning curve, and the first cell line (Pt.224) required several repeats before it was grown successfully. The first cell line to be characterized were Ascites from patient number 224 (Pt.224). These were grown for up to a month in concentrations of 5,10,20,50, and 100 cells per 20 microliter well for each of the three conditions (Pt.224 cells alone, CaMSCs alone as a control, and a co-culture of Pt.224 cells and CaMSCs). The cells in co-culture were grown with a 50:50 ratio of CaMSCs to Ascites for all cell lines. Each condition was tested using 7 wells patterned in alternate wells so that no two droplets were touching. Maintained in culture over a period of 14 days, we found that they formed spheroids under growth conditions in the 384 well hanging drop plates both with and without the presence of CaMSCs in co culture. The cells in co-culture were grown with a 50:50 ratio of CaMSCs to Ascites for all cell lines. Micrographs show that Pt. 224 cells in the lower culture densities were able to remain alive in culture for the entire 14 day period, but towards the end of that period some of the spheroids from the 50 and 100 cell conditions began to break up. The same was observed in CaMSCs and Co-cultures of the same densities. Alamar blue data for Pt. 224 was taken by [Student 1].

The second cell line to be characterized was Pt. 239 Ascites, which were grown for 7 days in 2,5,10,20, and 50 cells per well concentrations for Pt. 239 alone, Coculture, and CaMSC alone conditions. Spheroids formed in CaMSC and Co-Culture wells, but did not form in Pt. 239 alone. The experiment was repeated with the same results.

The third cell line was Pt. 240 Ascites which were grown for 7 days in 2,5,10,20, and 50 cell per well concentrations for Pt. 240, Coculture, and CaMSC conditions. The results were that spheroids did not form in the Pt. 240 ascites alone, however they did form for Co-Culture and CaMSC conditions. The experiment was also repeated with the same results. A third repeat of Pt 239 and Pt. 240, may be done in the upcoming semester to further corroborate this evidence. The shorter test period of Pt. 239 and Pt. 240 was due to the fragility of the spheroids that they formed, which began to fall apart around days 6-7 in the higher density cell concentrations.

Some problems that have been occurring over the semester have been delays because of training and scheduling conflicts as well as delays due to human error when preparing the cells to be plated that have required additional repeats to be done of the tests for the Pt. 224 plates.

In the next semester we intend to begin to harvest spheroids and characterize the Co-Culture and patient ascite spheroids further using fluorescent antibody tagging the CaMSCs and patient cells separately in order to differentiate them on fluorescent and confocal fluorescence micrographs. We will also be receiving additional patient cell lines to characterize and we plan to perform flow-cytometry to separate the different types of cells and determine how close our models are to what would naturally occur *in vivo*. The patient ascites that we have been using have not been flow-sorted, therefore, we hypothesize that the patient ascites contain the normal 0.01% MSCs that is normal and we want to test to see if this is the case.