While immuno-oncology-based therapies are rapidly becoming the treatment of choice for many tumor types, the assay systems to accurately test them in preclinical development are lacking. This is primarily due to the complexity and function of the immune system and its interaction with tumors in terms of cell types, cellular recruitment and organization, and microenvironment. The ultimate in vitro systems would 1. Combine patient specific tumor cells with autologous immune cells to counteract cytotoxicity due to interactions between allogeneic lymphocytes and tumor cells. 2. Be designed to force recruitment of lymphocytes and macrophages by the tumor cells and their microenvironment. 3. Promote macrophage polarization and lymphocyte activation that recapitulates the patient’s tumor by recapitulating the microenvironment, and 4. Result in tumor cell death that correlates to clinical response biomarker expression (PD-1, PD-L1, CTLA-4, etc.), and therapy mechanism of action. To develop these systems, we have focused our work on numerous areas with 2 presented here. 1. Checkpoint inhibitors, pembrolizumab and ipilimumab, in solid tumors such as ovarian cancer and melanoma, and 2. Lymphocyte and Macrophage migration, activation, and polarization in breast cancer. For checkpoint inhibitor studies, we have screened primary ovarian cancer tissues, melanoma, and matching lymphocytes for the expression of PD-1, PD-L1, and CTLA-4 by histology and flow cytometry. Both negative and positive tissues have then been utilized in 3D tissue models to examine the effects of drug upon the tumor cells and the other cell types within the model. We have seen correlation between Pembrolizumab binding to lymphocytes and PD-L1 expression and shown further correlation to the expression of PD-L1 on the matching tumor cells and drug efficacy. In breast cancer we have used our complex, multi-cell type models to examine macrophage and lymphocyte migration and recruitment into the tumor. We have also examined the polarization of macrophages in these systems and their impact upon tumor cell viability with the tumor cells promoting M2 macrophage polarization and the M2 macrophages promoting tumor cell viability compared to M1 macrophages. Secreted cytokines as measured by multiplex technology have also supported an immune protective environment with M2 macrophages as seen by changes in IL10, and TNFs compared to the presence of M1 macrophages. Our data to date reveals that these complex 3D in vitro models have the ability to recapitulate in vivo biology and biomarkers correlated drug response. These models can be used to both predict individual patient response to immuno-oncology agents and test new agents for efficacy in a preclinical setting.

Abstract

Drug Response Profiling in Melanoma without Cell Selection

Models

Immune Cell Migration In Vitro

Conclusions

- We can isolate and expand both tumor cells and immune cells without marker-based selection bias successfully in ovarian cancer, melanoma, and rare tumors.
- Pembrolizumab effectively blocks PD1 antigen sites in a dose dependent manner in isolated and expanded melanoma T-cells in 3D spherical cultures within 72 hours, We have also seen this result in ovarian cancer.
- We can track T-cell and macrophage migration within our 3D microtumors via multiphoton microscopy and flow cytometry.