Complex, 3D Tissues for Modeling the Immune Response in Cancer and Predicting the Activity of Immunotherapies

#5119

Teresa M. DesRochers[1], Lillia Holmes[1], Qi Guo[1], Lauren O'Donnell[1], Stephen Shuford[1], Larry Puls[2], Jeffrey Elder[2], Jeff Edenfield[2], Howland Crosswell[1]
[1]KIYATEC Inc.; Greenville, South Carolina 29605 USA | [2]Greenville Health System; Greenville, South Carolina 29605 USA |

Background

The immune system plays an active role in both the prevention and the promotion of cancer dependent upon its interaction with the tumor cells. The roles of both macrophages and T-cells in cancer progression have been heavily studied over the past few years. Macrophages have been found to be either tumor promoting or tumor preventing depending upon their differentiation status and the tumor microenvironment while the homing and cell destroying capabilities of T-cells have been manipulated to effect better, more specific tumor cell cytotoxicity through the development of therapies such as chimeric antigen receptor T-cells (CAR-T cells). Unfortunately the majority of research in the area of immune-oncology has relied upon either 2D cell culture or animal models. While a large amount of information has been learned from these models, it has been well established that 2D cell culture does not mimic in vivo biology and the immune system of mouse models differs from that of humans in numerous ways including T-cell subsets, cytokine receptors, and costimulatory molecule expression. To overcome these limitations, we have developed a number of 3D in vitro tissue models including multi-cell type models of glioblastoma (GBM), breast, and ovarian cancer.

Immune Tumor Models

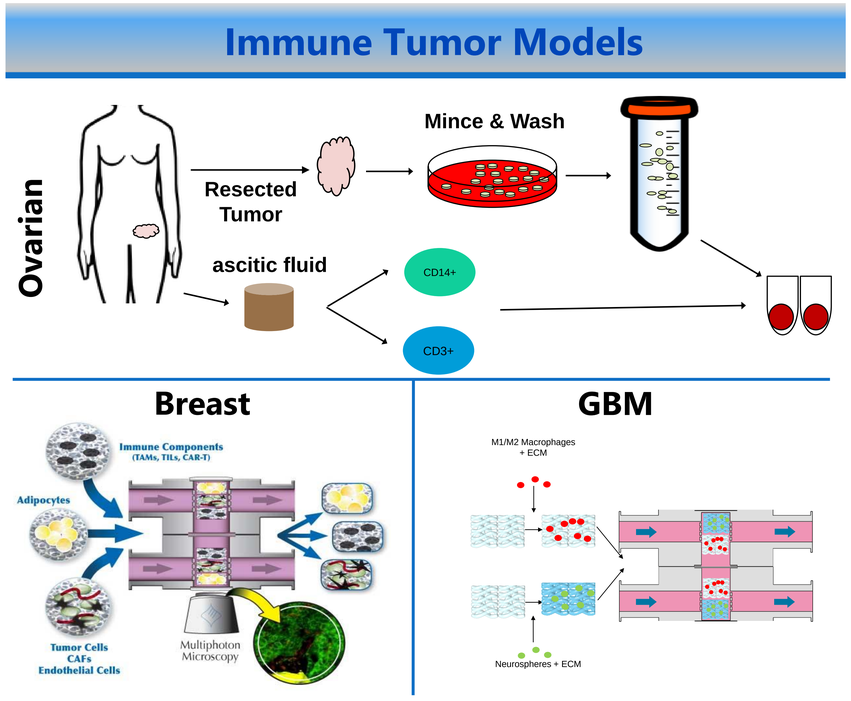


Figure 1: Model Development. (**Ovarian**) Both tumors and matched ascitic fluid are collected from patients. Tumor cells are then combined with immune cells in 3D spheroid assays. (**Breast**) Tumors and matched ascitic fluid are collected from patients. Tumor cells, endothelial cells, fibroblasts, and fat are then combined with immune cells in 3D microtumors. (**GBM**) Tumors are collected from patients and neurospheres are established. These are combined with differentiated macrophages from normal blood in 3D microtumors.

GREENVILLE HEALTH SYSTEM
INSTITUTE iTOR TRANSLATIONAL ONCOLOGY RESEARCH
With Special Thanks to the Biorepository

Ovarian – Immune Cells Affect Viability

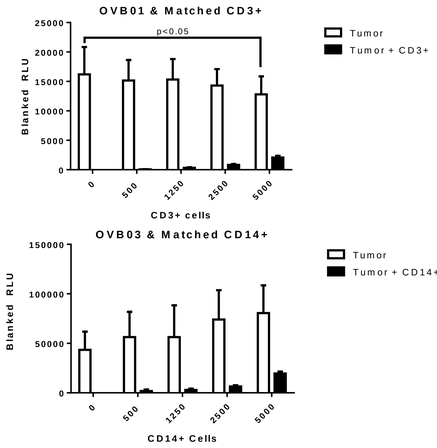
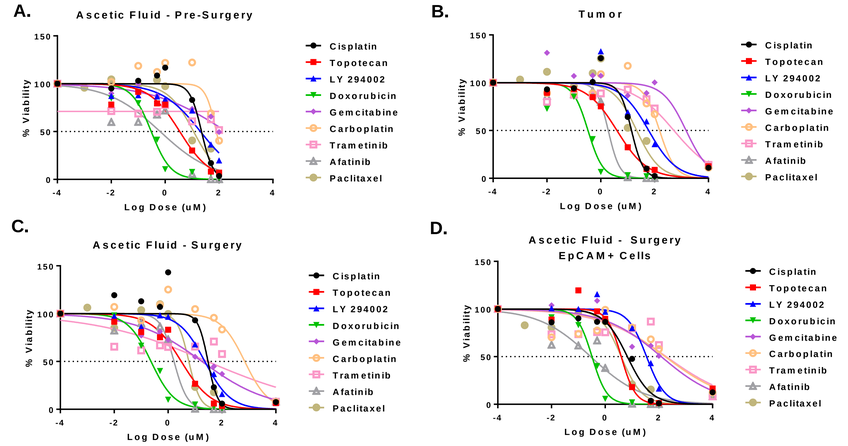


Figure 2: Immune cells co-cultured with patient-matched tumor cells can affect proliferation in a cell-type dependent manner. (**A**) When ovarian tumors cells were co-cultured with matched CD3+ cells for 96 hours, there was a significant drop if the viability of the tumor cell/CD3+ combo that was inconsistent with the viability of the CD3+ cells alone. (B) When ovarian tumor cells were co-cultured with matched CD14+ cells for 96 hours, viability of the tumor cell/CD14+ combos were consistent with the viability of the CD14+ cells alone.

Ovarian – Drug Response Profiling



Drug	IC50 (uM)			
	ascitic Fluid Pre-Surgery	Tumor	ascitic Fluid Surgery Unselected	ascitic Fluid Surgery EpCAM+
Cisplatin	22.45	13.91	30.34	6.716
Topotecan	3.493	3.964	3.097	3.976
LY 294002	20.47	60.35	23.14	34.82
Doxorubicin	0.3053	0.3359	0.2329	0.3238
Gemcitabine	>100	>100	18.15	>100
Carboplatin	79.72	>100	>100	>100
Trametinib	>100	>100	29	>100
Afatinib	0.654	1.901	1.664	0.2887
Paclitaxel	11.4	21.68	5.903	3.962

Figure 3: Drug response is affected by the source of the cells and the cell types present. ascitic fluid (**A, C, D**) and tumor (**B**) from the same patient were assessed for drug response in our EV3D DRP assay. The ascitic fluid was assessed both before surgery and at the time of surgery. Prior to surgery it predicted response to carboplatin however their was no response when the tumor and the ascitic fluid at surgery was assessed. The ascitic fluid at surgery predicted response to both Gemcitabine and Trametinib which was not predicted by the tumor. When the surgical ascitic fluid was selected for EpCAM+ cells (**D**) which made 20% of the cellular population, the drug response profile was more similar to the tumor than either unselected ascitic fluid samples, n = 6.

Glioblastoma

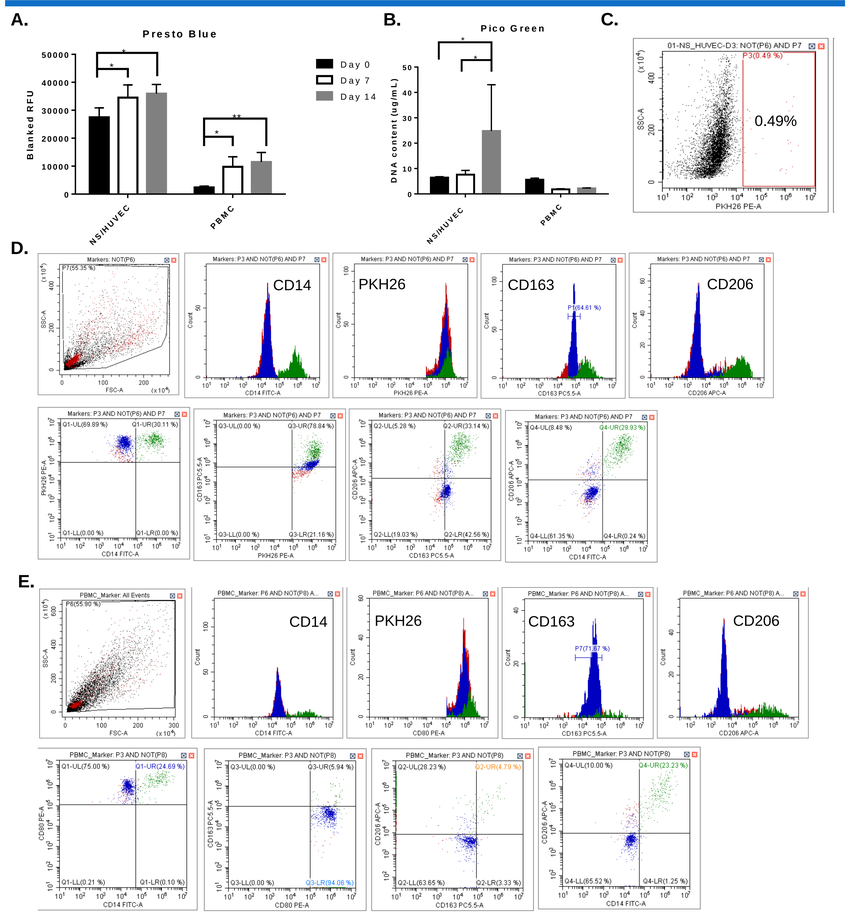


Figure 4: The presence of neurospheres and huvecs affects the differentiation of CD14+ PBMCs. Scaffolds containing NS/HUVEC or PBMCs were cultured together in perfusion for 14 days. Both metabolism (**A**) and cell numbers (**B**) increased in the NS/HUVEC scaffolds. However, while the PBMC scaffolds increased in metabolic activity, they lost cell numbers. The PBMCs were labeled with PKH26. After 1 week in culture a very small number of PKH26 cells were found in the NS/HUVEC scaffolds indicating a lack of cellular migration by the PBMCs (**C**). At day 7 (**D**) and day 14 (**E**), the PBMCs were removed from their scaffolds and examined for macrophage markers. At both days the cells were positive for both CD163 and CD206 but the signal was reduced at day 14 compared to day 7. *p<0.05, **p,0.01, error bars = standard deviation, n=3.

Conclusions

- The presence of CD3+ cells negatively affects cell viability in ovarian cancer while CD14+ cells do not
- Ascitic fluid of ovarian cancer patients affects drug response with EpCAM+ cells from ascitic fluid reflecting the response of the primary tumor.
- The different cell types of a tumor can affect the differentiation of CD14+ immune cells
- **Future Directions: preclinical immuno-oncology agent testing and predictive assay for checkpoint inhibitor responders**

This work is supported in part by NCI SBIR Contract #: HHSN261201300043C

KIYATEC®

KIYATEC Inc.| 900-B West Faris Road | Greenville | SC | 29605 www.KIYATEC.com