Development of a novel 3D model to investigate the role of heterogeneity in ovarian cancer

More than 70% of all patients diagnosed with epithelial ovarian cancer (EOC) succumb to their disease making EOC the most lethal gynecological cancer. The astonishingly high mortality rate of EOC can be explained largely by the fact that 75% of all cases are diagnosed at advanced or metastatic stages thus highlighting the urgency to better understand what cues in the primary tumor microenvironment (TME) promote metastasis. Up to 80% of these patients experience disease reoccurrence demonstrating that in addition to early events in EOC progression, how EOC populations acquire resistance to our current gold standard of treatment is poorly understood. While two-dimensional (2D) *in vitro* studies of ovarian cancer have provided fundamental insight, three-dimensional (3D) culture systems that more accurately mimic the *in vivo* TME in terms of biomechanical and biochemical stimuli, are critical research tools in studying ovarian cancer progression. To address this problem, we have developed a 3D culture model that allows for spatial and temporal control of cancer cell interactions with their TME. Our model has the capability to support 3D EOC growth in a mechanically relevant environment over at least a 4-week period while a physically separated fibroblast-rich matrix evolves under the influence of EOC secreted factors. This model maintains the spatial heterogeneity present in both the EOC growth, as well as the developing microenvironment. Using this model, we chose to address two critical gaps in knowledge in the progression of EOC, namely how heterogenous EOC populations acquire resistance and how EOC progression alters surrounding stromal cell populations to promote invasion and metastasis. Our work reveals that altering the dose and administration schedule of paclitaxel can produce heterogenous EOC populations that differ in the acquisition of single or multidrug resistance. By comparing these populations, we found that paclitaxel treatment induced nonuniform metabolic reprogramming across EOC populations which was crucial for the establishment of single and multidrug resistance. Our results provide insight into how current gold standard chemotherapies contribute to the development of chemoresistant EOC subpopulations and describe potential vulnerabilities in these cells that can be exploited for more effective treatment.