1493 DEVELOPING A 3D MULTICELLULAR MODEL TO INVESTIGATE STROMAL AND IMMUNE INTERACTIONS IN COLORECTAL CANCER TUMOUR MICROENVIRONMENT

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Background Colorectal cancer (CRC) is the 3rd most common cause of cancer related deaths worldwide.[1]Patients with stromal dense tumours (CMS4) have the worst disease-free progression survival rates and account for 23% of all CRC patients.[2] These patients have an inflamed immunophenotype and often do not respond well to current treatment options. Understanding cell-cell and cell-ECM interactions in CMS4 is necessary for developing new treatments for CMS4 patients.[3] We aim to develop a 3D model which replicates aspects of CMS4 CRC tumour microenvironment (TME) in order to study interactions taking place between colorectal cancer cells, stroma and immune cells in the TME of CRC.

Methods Spheroids were established from HCT116 and HT29 human CRC cell lines, and embedded into collagen type 1 hydrogels. To recapitulate stromal dense tumour microenvironments, bone marrow derived hMSCs were incorporated in the spheroids. Viability was analysed using calcein AM and propidium iodide staining. Using confocal microscopy and imageJ the extra cellular matrix of the spheroids was analysed. Finally, to mimic the immune landscape of CMS4 CRC patients, spheroids were co-cultured with Jurkat cells (T cell line) or activated T cells isolated from healthy patients. Using flow cytometry immune cell activation and polarisation in the 3D model was assessed.

Results Incorporating MSCs leads to reduced cell death and increased outgrowth from spheroids, mimicking the increased metastatic capacity of CRC in a stromal dense environment. Gels with hMSCs had significantly higher levels of fibronectin than gels with CRC cells alone. Co-culturing Jurkat cells with spheroids +hMSCs did not lead to a change in exhaustion markers compared to with cancer cells alone. Ongoing work of co-culturing PBMCs with CD3/CD28 beads will allow us to get a better understanding of the interactions between stromal cells and cytotoxic T cells in the TME of CMS4 CRC.

Conclusions Our 3D model provides a suitable tool for analysing interactions between CRC, stroma and immune cells, as well as cell-ECM interactions in CMS4 CRC. Our model mimics the high fibronectin levels present in CRC TME. As well as this, this model has the capabilities of being analysed at the single cell and whole gel level, assessing transcriptomic, intracellular, cellular and secreted proteins. This model also can also be used as a platform for screening immunotherapies. Making this an ideal model for examining cellular interactions and testing of novel therapeutics for CRC patients.

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