INFLAMMATORY TUMOUR MICROENVIRONMENT IN CRC ALTERS MACROPHAGE FUNCTION THROUGH MESENCHYMAL STROMAL CELL PD-1/PD-L1 SIGNALLING

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Background Colorectal cancer (CRC) is the 3rd leading cause of cancer related deaths worldwide despite recent improvement in treatment options. CRC patients with mesenchymal stromal cell (MSC) rich tumours (CMS4 subtype) have the lowest survival rates and show high levels of immunosuppression and treatment resistance. However, the role MSCs play in tumour promotion, in particular, their interaction with macrophages, which are the most abundant immune cell in CRC, is not fully understood.

Methods Bioinformatics analysis of 433 primary CRC tumours assessed by microarray was used to determine transcriptional changes in CMS4 changes. These transcriptional changes were then further investigated using complex 2D conditioning and co-culture systems in but mouse and human. Primary MSC were isolated from CRC patient tumours and expanded ex vivo. A 3D Gelatin methacrylate hydrogel model of CMS4 like tumours was developed. This 3D model combine HCT116 cancer cells, THP1 monocyte cell line and bone marrow derived MSC.

Results In stromal rich CMS4 tumours, we observed an enhanced TNF-α signalling signature. MSCs were conditioned with inflammatory tumour cells secretome (iTCS), and we observed an increased expression of PD-L1 and CD47 by both RNA-seq and flow cytometry. Analysis of primary CRC patient tumours revealed higher numbers of macrophages in the stromal compartment CRC. Conditioned MSCs were co-cultured with macrophages and reduced MHC-II and TNF-α expression, increased CD206 expression, and suppressed phagocytic function in macrophages was observed. Blocking PD-1, the receptors for PD-L1, on macrophages, restored macrophage phagocytosis levels in these cultures. Using the 3D CMS4 like model, we could show that addition of MSCs to the culture system increased the secretion of MIF, SerpinE1, IL-8, CXCL12 and CXCL1, increased the transcription of extracellular matrix remodelling genes while also increasing cancer cell expression of EGFR, supporting tumour growth and proliferation.

Conclusions These results show that tumour associated MSCs alter macrophage function and promote tumour immune evasion in 2D and 3D models. Using physiologically relevant models we show that stromal cells enhance cell surface and secreted immunomodulatory molecule expression in the tumour microenvironment. These findings and experimental models may suggest novel therapeutic targets for patients with MSC rich tumours and improve treatment stratification of patients for immunotherapy treatment.

Ethics Approval CRC patient samples were obtained from patients undergoing colon tumour resection at University Hospital Galway under an ethically approved protocol (Clinical Research Ethics Committee, Ref: C.A. 2074). Written informed consent was obtained from all patients prior to collection.

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