

immundominant SARS-CoV-2-specific CD8 T cell response revealed a regulated activation program that maintains CD8 T cell survival while halting their effector function and migratory capacity.

Conclusions The ORF1ab, that was found to be the source of an immunodominant SARS-Cov-2-specific CD8 T cell epitope, is not included in the majority of vaccine candidates in development, which may influence their clinical activity. Furthermore, these data may be a cautious indication that SARS-CoV-2 specific CD8 T cells – unlike CD4 T cells – are less likely to contribute to the immunopathology observed in severely and critically ill COVID-19 patients.

Ethics Approval The samples from both COVID-19 patients were collected in accordance with the Declaration of Helsinki after approval by the institutional review boards.

Consent Each participant signed informed consent.

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Tumor and stromal cell biology

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THE MESENCHYMAL STROMAL COMPARTMENT IN COLORECTAL CANCER GREATLY ALTERS THE INNATE TUMOUR IMMUNE MICROENVIRONMENT BOTH IN 2D AND 3D CULTURE SYSTEMS

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Background Colorectal cancer is the fourth most common occurring cancer and despite new treatment options it remains the third leading cause of cancer related deaths worldwide.¹ Of the four Consensus Molecular Subtypes (CMS), the mesenchymal stromal rich CMS4 tumours are shown to have the worst disease free progression survival. However the role mesenchymal stromal cells (MSC) play in the tumour immune microenvironment has yet to be fully elucidated. Understanding the complex communication in this stromal cell rich multicellular environment is challenging but may reveal novel targets for the treatment of colorectal cancer patients.

Methods Tumour cell secretome (TCS) was generated from colon cancer cells with/without the addition of TNF- α , an inflammatory stimulus using both human and mouse cell lines. MSCs were then conditioned with the TCS and inflammatory TCS and changes in surface and secreted immunomodulatory molecules were assessed using RNA-seq, flow cytometry and ELISA analysis. Macrophage antigen processing and migration following co-culture with the TCS conditioned MSCs was observed using DQ-ova and transwell experiments. A Gelatin Methacryloyl hydrogel, 3D triple culture systems was established to study the role of MSC in the colon tumour immune microenvironment. HCT116 colon cancer cell line with THP1 monocytic cell line and primary bone marrow derived MSCs were embedded in the hydrogel and incubated for 10 days, changing the media on Day 8 with/without the addition of TNF- α . Cell proliferation viability and protein secretion were assessed from the 3D CRC system.

Results Bioplex analysis revealed secretion of potent chemokines and cytokines from the cancer cells. This inflammatory TSC resulted in increased expression of cell surface MSC immunomodulatory markers PD-L1 and CD47 and a variety of secreted molecules. These conditioned MSCs reduced macrophage-mediated antigen processing and increase monocyte migration. A triple culture 3D model of CRC was successfully developed, and while the addition of MSC to the system did not alter spheroid size they increased the release of potent chemokines (CCL2, CXCL12), cytokines (IL-6, IL8) and growth factors (GM-CSF) from the culture system.

Conclusions The inflammatory tumour cell secretome can alter MSC surface expression and secretion of a variety of immunomodulatory makers. These tumour conditioned MSCs can alter innate immune cell antigen processing and migration. When MSCs are combined in 3D with monocytes and colon cancer cells the MSC significantly alter the secretion of immune modulating and tumour promoting factors from the culture system. Targeting MSC immune suppression in the colon tumour microenvironment could be a novel therapeutic target.

Ethics Approval Human MSC (hMSC) were isolated from the bone marrow of three healthy volunteers at Galway University Hospital under an ethically approved protocol (NUIG Research Ethics Committee, Ref: 08/May/14) according to a standardized procedure.

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SUGAR HIGH: DOES THE SIALIC ACID PROFILE OF CANCER-ASSOCIATED FIBROBLASTS INDUCE A MORE TUMOUR-PERMISSIVE MICROENVIRONMENT?

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Background Immunosuppressive tumour microenvironments (TME) inhibit the effectiveness of cancer immunotherapies. Sialic acids, which exist as terminal sugars of glyco-conjugates, are highly expressed on cancer cells and are involved in various pathological processes including increased immune evasion, tumour invasiveness and tumour cell metastasis.¹ Siglecs (Sialic acid-binding immunoglobulin-type lectins) are expressed on immune cell surfaces and bind sialic acid. Siglec binding to hypersialylated tumour glycans blocks immune cell activation to promote immunosuppression.¹ ²Intestinal stromal cells (iSCs), precursors to cancer-associated fibroblasts (CAFs), are a key component of the TME and play a vital role in tumour progression by enhancing a tumour-promoting microenvironment. The aim of this study was therefore to investigate if iSC/CAF sialylation contributes to enhanced immunosuppression in the TME.

Methods iSCs were isolated from colorectal cancer patient biopsies and cultured ex vivo. Informed consent was obtained from all patients prior to sampling. Tumour-derived iSCs were termed CAFs while control iSCs, isolated from tumour-adjacent non-cancerous tissue, were termed normal-associated

fibroblasts (NAFs). NAFs/CAFs were then co-cultured with healthy allogeneic PBMCs and their immunosuppressive properties were assessed by flow cytometry.

Results CAFs significantly suppressed the proliferation of CD8+ and CD4+ T-cells and induced a more exhausted T-cell phenotype as evidenced by increased expression of the exhaustion markers TIM-3, LAG-3 and PD-1 when compared to co-culture with control NAFs, thereby demonstrating their potent immunosuppressive properties. Strikingly, CAFs also induced significantly higher expression of both Siglec-7 and Siglec-9 receptors on CD8+ T-cells specifically.

To elucidate the role of sialylation on CAF-mediated immunosuppression, NAFs/CAFs were treated with the sialyltransferase inhibitor (SI) P-3FAX-Neu5Ac prior to co-culture. Reduction of sialic acid expression on NAFs/CAFs was confirmed by flow cytometry and the SI-treated NAFs/CAFs were then co-cultured with allogeneic T-cells to assess the functional consequences of reduced NAF/CAF sialylation. SI-treated CAFs induced significantly less CD4+TIM-3+ and both CD4+LAG-3+ and CD8+LAG-3+ T-cells compared to their untreated counterparts. Interestingly, SI-treated CAFs also induced significantly less Siglec-7 and -9 receptor-expressing CD8+ T-cells.

Conclusions These results demonstrate that non-haematopoietic stromal cells in the tumour-microenvironment can suppress activated T-cells and that this immunosuppressive effect can be significantly reversed through the modulation of sialylation on the stromal cell surface. These results support the hypothesis that stromal cell sialylation plays a role in their immunosuppressive properties. Understanding how sialylation of stromal cells is regulated and functions to enhance immunosuppression in the TME could uncover novel immune checkpoints to reactivate anti-tumour immunity, allowing for tumour cell clearance.

Ethics Approval This study was approved by Galway University Hospitals' Clinical Research Ethics Committee, approval number C.A 2074.

Consent N/A

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DISTINCT GENOMIC FEATURES ACROSS CYTOLYTIC SUBGROUPS IN SKIN MELANOMA

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Background Skin melanoma is a highly immunogenic cancer. The intratumoral immune cytolytic activity (CYT) reflects the ability of cytotoxic T cells and NK cells to eliminate cancer cells, and is associated with improved patient survival. Despite the enthusiastic clinical results seen in advanced-stage metastatic melanoma patients treated with immune checkpoint inhibitors (ICI), a subgroup of them will later relapse and develop acquired resistance. We questioned whether CYT associates with different genomic profiles in skin melanoma.

Methods We explored the TCGA-SKCM dataset and stratified patients to distinct subgroups of cytolytic activity. We calculated the tumor immune contexture, somatic mutations, recurrent copy number aberrations, chromothripsis, cancer neoepitopes, immunophenoscore, mutational signatures, kataegis and strand asymmetry in each cytolytic subgroup.

Results CYT was higher in enriched in immune-related gene sets metastatic tumors. Distinct mutational and neoantigen loads, primarily composed of C>T transitions, along with specific types of copy number aberrations, characterized each cytolytic subgroup. More chromothripsis events were found across CYT-low tumors SBS7a/b, SBS5 and SBS1 were the most prevalent mutational signatures in both cytolytic subgroups, but SBS1 differed significantly between them. SBS7a/b were mutually exclusive with SBS5 and SBS1 in both CYT subgroups. Mutational strand asymmetries related to the processes of DNA transcription and replication differed between CYT-high and CYT-low tumors. CYT-high patients had markedly higher immunophenoscore and should consequently, display an expected clinical benefit compared to CYT-low patients who either received or not, ICI.

Conclusions Our data highlight the existence of distinct genomic features across cytolytic subgroups in skin melanoma patients, which could affect their relapse rate or resistance to ICI.

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PRELP-FACILITATED ENHANCEMENT OF MHC CLASS I SURFACE EXPRESSION IN B16F10 MELANOMA CELLS

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Background PRELP (proline arginine-rich end leucine-rich repeat protein; also Prolargin), a small leucine-rich proteoglycan, functions as a molecule anchoring basement membranes to connective tissues via the interaction with collagens and heparin. PRELP facilitates the binding of cells to glycosaminoglycans as an important regulator of cell adhesion and thus displays pathophysiological features. Melanoma is an immunogenic tumor, whose relationship with immune cells resident in the microenvironment significantly influences cancer cell proliferation, progression and metastasis. Evasion from immune surveillance is a hallmark of melanoma progression. While our laboratory reported that the proteoglycan biglycan (BGN) was enhancing MHC class I in tumor cells,¹ the role of PRELP in tumor immunology has not been studied.

Methods The murine metastatic melanoma cell line B16F10, characterized by a reduced expression of MHC class I surface antigens was chosen for this study. B16F10 cells were transiently transfected with PRELP as well as co-transfected with BGN. Expression of antigen processing machinery (APM) components and PRELP was determined by qPCR and MHC class I surface expression by flow cytometry. Promoter activity of APM components was analysed by luciferase reporter assays. XTT assays were used to determine cell proliferation. The association of PRELP and MHC class I was studied by bioinformatics in a mixed melanoma dataset of 83 samples.²

Results Over-expression of PRELP in B16F10 cells enhanced the expression of MHC class I surface antigens, which was