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

Results CAFs significantly supressed 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

REFERENCES

1. Wang L, Liu Y, Wu L, Sun XL. Sialyltransferase inhibition and recent advances. *Biochim Biophys Acta* 2016 Jan;1864(**1**):143-53.
2. Munkley J, Scott E. Targeting aberrant sialylation to treat cancer. *Medicines (Basel)* 2019 Oct 13;**6**(4):102.

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867 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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868 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