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

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867 DISTINCT GENOMIC FEATURES ACROSS CYTOLYTIC SUBGROUPS IN SKIN MELANOMA

[1]Apostolos Zaravinos*, [2]Constantinos Roufas, [3]Ilias Georgakopoulos-Soares. *[1]Qatar University; [2]European University Cyprus, Nicosia, Cyprus; [3]University of California, San Francisco, San Francisco, CA, USA*

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

Helene Schäfer*, Karthikeyan Subbarayan, Barbara Seliger. *Martin-Luther-University Halle-Wittenberg, Medical Faculty, Halle (Saale), Germany*

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

due to a PRELP-mediated transcriptional upregulation of components of the MHC class I APM components TAP1, TAP2 and TAPBP as determined by qPCR and promoter assay in PRELP transfectants versus mock controls. Furthermore, MHC class I surface expression was even more pronounced upon BGN co-transfection with PRELP. PRELP overexpression was able to inhibit the proliferation of the B16F10 cells. Bioinformatics analyses demonstrated a positive correlation of PRELP with HLA-A , -B and -C alleles in human melanoma.

Conclusions Our findings demonstrated that overexpression of PRELP correlates with higher MHC class I expression and inhibits cell proliferation. For the first time, co-transfections of the two proteoglycans PRELP and BGN had a synergistic effect on upregulating MHC class I expression. Therefore, PRELP can serve as a novel therapeutic strategy that deserves further investigation.

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869 ANTI-LUNX TARGETING THERAPY FOR LUNG CANCER

Xiaohu Zheng*, Weihua Xiao, Zhigang Tian. *USTC*

Background The identification of novel therapeutic targets in lung cancer for the generation of targeted drugs is an urgent challenge. Lung-specific X (LunX) is a member of the palate, lung, and nasal epithelium clone (PLUNC) protein family. Some reports have suggested that the human PLUNC gene (also named LUNX) might be a potential marker for NSCLC, and PLUNC mRNA has been identified in peripheral blood and mediastinal lymph nodes from NSCLC patients.It is unclear whether LunX expression is associated with the pathological type and pathological severity in lung cancer patients. The utility of LunX as a potential therapeutic target in NSCLC is uncertain.

Methods Clinically, 80% of lung cancers are non-small-cell lung cancers (NSCLCs). Here, we analyzed 158 NSCLC samples and detected LunX expression.

Results It showed that the expression of LunX were elevated in 90% (108/150) lung cancers by IHC staining, which accompanied with significantly lower rate of postsurgery survival. Further evaluation of LunX expression in invasive tumor cells in subclavicular lymph nodes, draining lymph nodes, hydrothorax of lung cancer patients, turned out that LunX is highly expressed in invasive lung cancer cells. These data indicated that LunX overexpresses in lung cancer and associates with tumorigenesis and tumor progression.

Mechanistically, we discovered that LunX bound to 14-3-3 protein and facilitated their activation by maintaining these proteins in a dephosphorylated state, thereby contributing to the activation of pathways downstream of 14-3-3 protein, such as the Erk1/2 and JNK pathways. Thus, LunX promoted tumor growth and metastasis.

Furthermore, we generated a therapeutic antibody specific for lung cancer, which not only inhibited lung cancer growth and reduced Ki67 staining and angiogenesis in xenograft model of subcutaneously transplanted tumor, but also blocked tumor metastasis and invasion, improved the survival of these mice. We also detected that antibody treatment induces LunX antigen-antibody complex endocytosis and the degradation of LunX protein.

Conclusions Our study suggests that LunX is a novel therapeutic target in lung cancer and that the LunX-targeted therapeutic antibody may have considerable clinical benefit.

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870 INVESTIGATING SEXUAL DIMORPHISM IN THE TUMOR IMMUNE MICROENVIRONMENT OF NON-MUSCLE INVASIVE BLADDER CANCER

[1]Stephen Chenard*, [1]Chelsea Jackson, [2]Thiago Vidotto, [1]Celine Hardy, [1]Tamara Jamaspishvilli, [1]David Berman, [3]David Robert Siemens, [1]Madhuri Koti. *[1]Queen's University, Kingston, ON, Canada; [2]Johns Hopkins School of Medicine, Baltimore, MD, USA; [3]Kingston Health Sciences Centre, Kingston, Canada*

Background While the incidence of non-muscle invasive bladder cancer (NMIBC) is four times higher in men than women, female patients display earlier recurrence than their male counterparts following treatment with Bacillus Calmette-Guerin (BCG) immunotherapy.[1] While patient sex (biological differences) and gender (social/behavioral differences) have long been associated with NMIBC incidence and clinical outcome, these factors remain the most understudied phenotypes in biomarker and treatment design.[2] We hypothesized that sexual dimorphism in the pre-existing tumor immune microenvironment (TIME) may contribute to the poor clinical outcomes observed in female NMIBC patients.

Methods To test this hypothesis, we interrogated the expression patterns of genes associated with specific immune cell populations and immune checkpoint pathways using tumor transcriptome profiles from n=460 NMIBC patients (357 males and 103 females). Based on this interrogation, we utilized multiplex immunofluorescence to selectively evaluate the density and spatial distribution of CD79a+ (B), CD163+ (M2-like tumor associated macrophages), and PD-L1+ (programmed death ligand 1) cells in an independent cohort of 510 NMIBC tumors collected from n=390 patients (305 males and 85 females).

Results We observed significantly higher expression of immune checkpoints genes CTLA4, PDCD1, TIGIT, LAG3 and ICOS in tumors from female patients. Importantly, transcript levels of the B cell recruiting chemokine CXCL13 and the B cell surface molecule CD40 were significantly increased in tumors from female patients. Multiplex immunofluorescence revealed that CD163+ cells were significantly higher in epithelial and stromal compartments of high-grade tumors ($p = 0.0011$, $p = 0.00034$, respectively) from female patients compared to males. While no sex-associated differences were observed in the density of CD79a+ B cells, this population was found to be significantly increased in the epithelial and stromal compartments ($p = 6.9e-9$, 9.4e-10, respectively) of high-grade tumors compared to low-grade tumors. PD-L1 expression was significantly higher in the epithelial compartment of high-grade tumors from female