1444 TARGETING SIALIC ACID ON STROMAL CELLS REVERSES T CELL SUPPRESSION IN THE COLORECTAL TUMOUR MICROENVIRONMENT A NEW TUMOUR STROMAL CELL IMMUNE CHECKPOINT

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Background Hypersialylation of cancer cells induces an immunosuppressive microenvironment. The binding of sialic acid by siglec receptors expressed on immune cells initiates a downstream response via immunoreceptor tyrosine-based inhibitory motif (ITIM) signalling. Cancer associated fibroblasts (CAFs) in the colorectal cancer (CRC) microenvironment are highly immunosuppressive and associated with poor survival. The role of sialyation in stromal cell-mediated immunosuppression, however, is unknown. Here, we investigated if sialylation of CAFs contributed to their potent immunosuppressive properties.

Methods Tumour cell secretome (TCS) from multiple CRC cell lines was used to condition primary human and mouse bone marrow-derived stromal cells, as CAF precursors. Normal and CAFs were isolated from colon tumour resections. Stromal cells were cultured with stimulated splenocytes (mouse) or PBMCs (human) and their immunosuppressive properties were assessed by flow cytometry. An in vivo mouse model of CT26 CRC was used to assess the role of highly sialylated stromal cells in tumour development. Mice were injected subcutaneously with CT26 cells alone, or co-injected with TCS-conditioned stromal cells, either control or de-sialylated. 14 days post induction, tumous, draining lymph nodes and spleen were assessed for frequency and expression of T cell activation markers.

Results Tumour conditioning resulted in significantly higher expression of both α2,6-linked sialic acid and specific Siglec ligands on stromal cells. CAFs were significantly more sialylated than stromal cells isolated from adjacent normal associated tissue (NAFs) (figure 1). Following co-culture, CAFs induced significantly higher levels of CD8+ T cells with an exhausted phenotype as determined by TIM-3 and PD-1 expression. Siglec-7 and -9 receptors were induced by CAFs on CD8 T cells. Furthermore, de-sialylation of CAFs, specifically, prior to co-culture resulted in a significant reduction in exhausted CD8+ T cells and attenuation of their immunosuppressive ability (figure 2).

14d post tumour induction in vivo, mice with TCS-conditioned, stromal-dense tumours had significantly fewer activated CD4+ and CD8+ CD25-expressing T cells, both intratumourally and distally in the draining lymph nodes and spleen. They also had low levels of cytotoxic granzyme B-expressing CD8+ T cells. Interestingly, this suppression was sialylation-dependent. De-sialylation of TCS-conditioned stromal cells led to restoration of activated T cell levels in tumours and peripheral lymphoid tissues, as well as a marked increase in cytotoxic T cells (figure 3).

Conclusions These results demonstrate, for the first time, that tumour stromal cells suppress activated T cells through sialic acid dependent interactions. We show that targeting stromal cell sialylation may represent a novel immune checkpoint to reactivate anti-tumour immunity.

Ethics Approval The animal study was approved by the Animals Care Research Ethics Committee of the National University of Ireland, Galway (NUIG) and conducted under individual and project authorisation licenses from the Health Products Regulatory Authority (HPRA) of Ireland (AE19125/P077). The study using human samples was approved by University Hospital Galway Ethics committee under an ethically approved protocol (Clinical Research Ethics Committee, Ref: C.A. 2074).

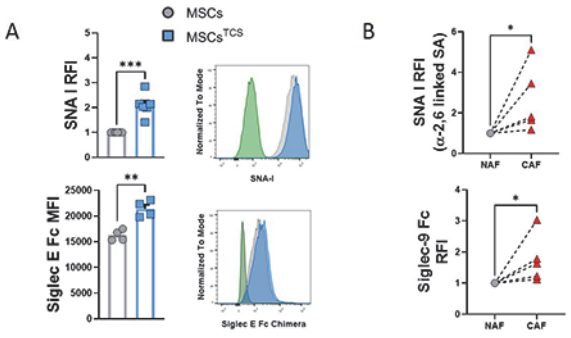

Figure 1. Exposure of stromal cells to tumour cell secretome or microenvironment increases sialic acid and specific Siglec ligand expression. (A) Mouse bone marrow-derived mesenchymal stromal cells (MSCs) have elevated SNA I (α2,6 sialic acid) and Siglec E ligand expression following exposure to tumour cell secretome from CT26 murine colon cancer cells (MSC^{TCS}). Isotype control is shown as green histogram. (B) Cancer-associated fibroblasts (CAFs) isolated from colorectal cancer patient biopsies express higher levels of SNA I and Siglec 9 ligand compared to fibroblasts isolated from non-cancerous (NAF), adjacent intestinal tissue. Data are mean ± SD; *p < 0.05, **p < 0.01, and ***p < 0.001 using (A) unpaired t-test and (B) ratio paired t-test. n = 4 biological replicates.

Abstract 1444 Figure 1 Exposure of stromal cells to tumour cell secretome or microenvironment increases sialic acid and specific Siglec ligand expression. (A) Mouse bone marrow-derived mesenchymal stromal cells (MSCs) have elevated SNA I (α2,6 sialic acid) and Siglec E ligand expression following exposure to tumour cell secretome from CT26 murine colon cancer cells (MSCTCS). Isotype control is shown as green histogram. (B) Cancer-associated fibroblasts (CAFs) isolated from colorectal cancer patient biopsies express higher levels of SNA I and Siglec 9 ligand compared to fibroblasts isolated from non-cancerous (NAF), adjacent intestinal tissue. Data are mean ± SD; *p < 0.05, **p < 0.01, and ***p < 0.001 using (A) unpaired t-test and (B) ratio paired t-test. n = 4 biological replicates.

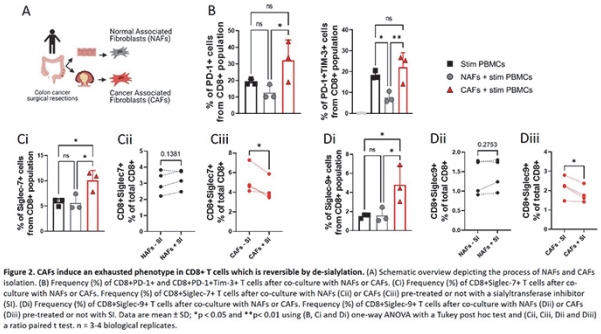

Figure 2. CAFs induce an exhausted phenotype in CD8+ T cells which is reversible by de-sialylation. (A) Schematic overview depicting the process of NAFs and CAFs isolation. (B) Frequency (%) of CD8+PD-1+ and CD8+PD-1+Tim-3+ T cells after co-culture with NAFs or CAFs. (Ci) Frequency (%) of CD8+Siglec-7+ T cells after co-culture with NAFs or CAFs. Frequency (%) of CD8+Siglec-7+ T cells after co-culture with NAFs (Cii) or CAFs (Ciii) pre-treated or not with a sialyltransferase inhibitor (SI). (Di) Frequency (%) of CD8+Siglec-9+ T cells after co-culture with NAFs or CAFs. Frequency (%) of CD8+Siglec-9+ T cells after co-culture with NAFs (Dii) or CAFs (Diii) pre-treated or not with SI. Data are mean ± SD; *p < 0.05 and **p< 0.01 using (B, Ci and Di) one-way ANOVA with a Tukey post hoc test and (Cii, Ciii, Dii and Diii) a ratio paired t test. n = 3-4 biological replicates.

Abstract 1444 Figure 2 CAFs induce an exhausted phenotype in CD8 + T cells which is reversible by de-sialylation. (A) Schematic overview depicting the process of NAFs and CAFs isolation. (B) Frequency (%) of CD8+PD-1+ and CD8+PD-1+Tim-3+ T cells after co-culture with NAFs or CAFs. (Ci) Frequency (%) of CD8+Siglec-7+ T cells after co-culture with NAFs or CAFs. Frequency (%) of CD8+Siglec-7+ T cells after co-culture with NAFs (Cii) or CAFs (Ciii) pre-treated or not with a sialyltransferase inhibitor (SI). (Di) Frequency (%) of CD8+Siglec-9+ T cells after co-culture with NAFs or CAFs. Frequency (%) of CD8+Siglec-9 + T cells after co-culture with NAFs (Dii) or CAFs (Diii) pre-treated or not with SI. Data are mean ± SD; *p < 0.05 and **p< 0.01 using (B,

Ci and Di) one-way ANOVA with a Tukey post hoc test and (Cii, Ciii, Dii and Diii) a ratio paired t test. n = 3-4 biological replicates

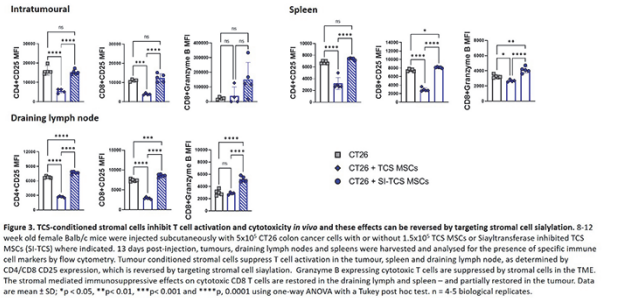

Figure 3. TCS-conditioned stromal cells inhibit T cell activation and cytotoxicity *in vivo* and these effects can be reversed by targeting stromal cell sialylation. 8-12 week old female Balb/c mice were injected subcutaneously with 5x10^5 CT26 colon cancer cells with or without 1.5x10^5 TCS MSCs or Siayltransferase inhibited TCS MSCs (SI-TCS) where indicated. 13 days post-injection, tumours, draining lymph nodes and spleens were harvested and analysed for the presence of specific immune cell markers by flow cytometry. Tumour conditioned stromal cells suppress T cell activation in the tumour, spleen and draining lymph node, as determined by CD4/CD8 CD25 expression, which is reversed by targeting stromal cell siaylation. Granzyme B expressing cytotoxic T cells are suppressed by stromal cells in the TME. The stromal mediated immunosuppressive effects on cytotoxic CD8 T cells are restored in the draining lymph and spleen – and partially restored in the tumour. Data are mean ± SD; *p < 0.05, **p< 0.01, ***p< 0.001 and ****p, 0.0001 using one-way ANOVA with a Tukey post hoc test. n = 4-5 biological replicates.

Abstract 1444 Figure 3 TCS-conditioned stromal cells inhibit T cell activation and cytotoxicity in vivo and these effects can be reversed by targeting stromal cell sialylation. 8-12 week old female Balb/c mice were injected subcutaneously with 5x105 CT26 colon cancer cells with or without 1.5x105 TCS MSCs or Siayltransferase inhibited TCS MSCs (SI-TCS) where indicated. 13 days post-injection, tumours, draining lymph nodes and spleens were harvested and analysed for the presence of specific immune cell markers by flow cytometry. Tumour conditioned stromal cells suppress T cell activation in the tumour, spleen and draining lymph node, as determined by CD4/CD8 CD25 expression, which is reversed by targeting stromal cell siaylation. Granzyme B expressing cytotoxic T cells are suppressed by stromal cells in the TME. The stromal mediated immunosuppressive effects on cytotoxic CD8 T cells are restored in the draining lymph and spleen – and partially restored in the tumour. Data are mean ± SD; *p < 0.05, **p< 0.01, ***p< 0.001 and ****p, 0.0001 using one-way ANOVA with a Tukey post hoc test. n = 4-5 biological replicates.

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