TARGETING STROMAL CELL SIALYLATION TO OVERCOME TUMOUR-INDUCED IMMUNOSUPPRESSIVE EFFECTS ON NK CELLS IN COLORECTAL CANCER

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Background Resistance to anti-cancer therapeutics in colorectal cancer (CRC) is high. A molecular subtype of CRC, CMS4, has a high proportion of mesenchymal stromal cells (MSCs) in the tumour microenvironment (TME), which are associated with cancer progression. Sialylation is a post-translational modification where sialic acids are added to glycoproteins. These sialic acids bind immune cell inhibitory receptors called Siglecs, such as Siglecs 7 and 9 on natural killer (NK) cells, which when bound signal through ITIM motifs to inhibit immune activation. We have shown in our laboratory that stromal cell sialylation is increased in CRC and inhibits anti-cancer effector functions.

Methods To determine the level of expression and functional role of sialylation in stromal cell immunosuppression, we use histological analysis of CRC patient tissue, probing for Siglec 7 and 9 ligands. To assess immunomodulatory effects of stromal cells on immune cells we ran co-culture assays in a human ex vivo model of CRC. We examined the effects of stromal cell sialylation on NK cell function, using sialic acid targeting reagents (SI). Flow cytometric analysis of Siglec receptor expression and functional cytotoxicity of CMS4 CRC cells are employed post co-culture. We also performed a mouse in vivo study of stromal-rich CRC incorporating SI treatment.

Results Immunofluorescent staining of patient CRC tissue showed strong antigen co-localization of stromal marker α-SMA and Siglec 9 ligand, indicating upregulated sialylation in CRC stroma. Flow cytometric analysis of ex vivo cancer-associated fibroblasts (CAFs) and normal-associated fibroblasts (NAFs) showed significantly higher Siglec 7 and 9 ligand expression in CAFs versus NAFs. Primary human NK cells post co-culture with CAFs showed upregulation of Siglec 9 receptor expression which was reduced when stromal cells were pre-treated with sialic acid removing reagents. Functional cytotoxicity assays showed decreased cytotoxic capacity of NK cells when exposed to CAFs, which was increased with CAF sialic acid removal.

In vivo mouse model showed elevated Siglec E and Siglec G receptor expression in NK cell subsets of tumour and draining lymph nodes, which are orthologues of Siglec 7 and 10. This expression was dramatically reduced with sialyltransferase inhibitor treatment of MSCs.

Conclusions Together this data shows targeting stromal cell sialylation in our models of stromal-rich CRC TME increases NK cell cytotoxicity, reduces NK cell inhibitory receptor expression, providing evidence that sialylation has the potential to impact immune cell responses in the TME.

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REFERENCES

Ethics Approval Ethical approval was obtained from the HPRA with project and individual authorizations for animal studies.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal

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