Impact of heparan sulphate binding domain of chemokine CCL21 to migration of breast cancer cells

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Introduction

Lymph node metastasis constitutes a key event in breast cancer progression. Chemokines are small proteins, which can promote metastatic spread by inducing cancer cell migration and invasion. Chemokine function is dependant upon their binding to both cell surface heparan sulphate (HS) molecules and to their specific receptor. Our group has demonstrated a significant increase in chemokine receptor CCR7 expression in cancerous breast epithelia compared to healthy controls.

Aim

To test the hypothesis that a non-HS binding forms of chemokine CCL21 can disrupt the normal response to CCL21, therefore reducing the metastasis of CCR7-expressing cancer cells.

Methods

- Synthesis of truncated CCL21 chemokine (Δ98-134 c-terminal basic extension) to investigate a possible linkage between chemokine binding capacity and cell activation.
- Test the ability of Wild type (WT) and mutant-CCL21 to stimulate a dose-dependent increase in intracellular-free calcium in PBMC and breast cancer epithelial cells MDA-MB-231.
- Determine the ability of CCL21 to stimulate chemotaxis within a concentration gradient and compare the potential of WT and mutant-CCL21 to stimulate the migration of cells across endothelium.
- Determine the potential of mutant CCL21 for therapeutic blockade of the migration of Breast Cancer cells *in vivo*.

Conclusions

- There were a significant increase in chemokine receptor CCR7 expression in cancerous breast epithelia compared to healthy controls and in MDA-231 cells compared to primary Breast epithelial cells. Mutant-CCL21 at concentrations 5 and 10nM showed potential to mobilise Ca2+ at levels similar to that produced by WT-CCL21.
- Mutant-CCL21 stimulated no increased in transendothelial cell migration of MDA-MB-231 compared to WT-CCL21.
- Mutant CCL21 in xenograft brain tumor models showed a substantial inhibition of tumour growth compared to WT-CCL21.

Results:

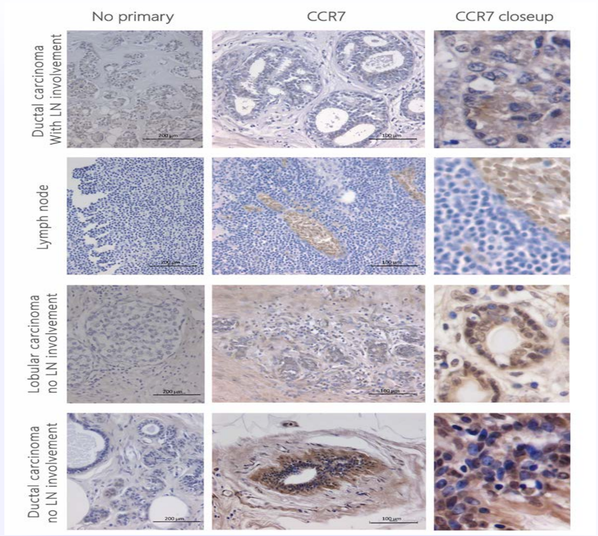

Figure 1: CCR7 staining in human breast cancer tissue

Immunohistochemical labelling of paraffin embedded healthy from human invasive breast cancer were stained for anti-human CCR7 at 20x magnification. No primary antibody was used as a control.

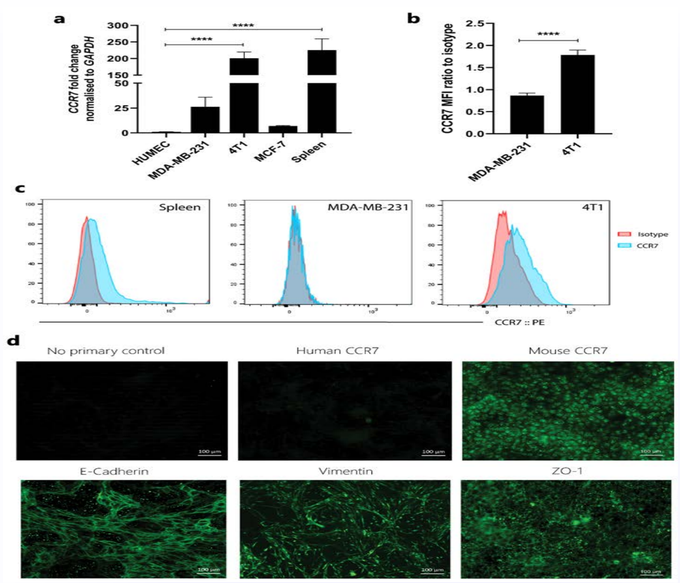

Figure 2: CCR7 expression in breast cancer cell lines

a) CCR7 expression was assessed at RNA in several breast cancer cell lines using RT-PCR. **b)** CCR7 expression at protein level. **c)** Confirmation of CCR7 antibody inter-species specificity, murine spleen, MDA-MB-231 and 4T1 cells. **D)** CCR7 expression in 4T1 cells was further confirmed using Immunofluorescence.

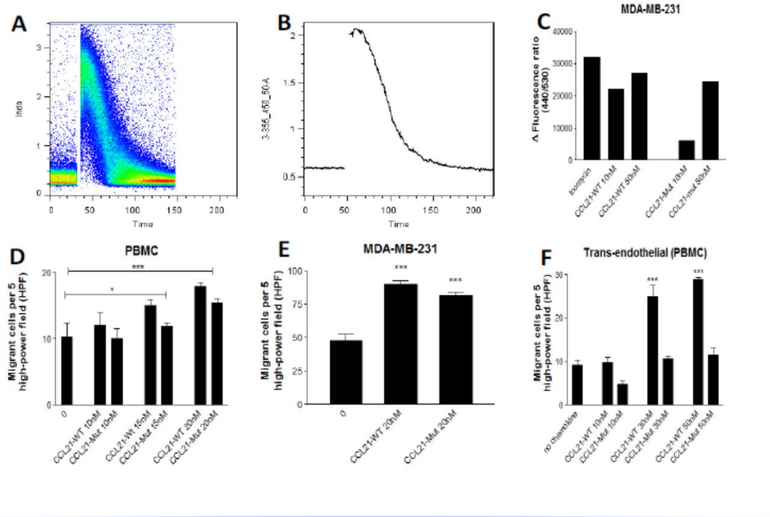

Figure 3: Mechanism of action of the non-glycosaminoglycan-binding CCL21 mutants

Calcium flux results: (A&B), Representative changes in the intracellular Ca2+ concentration immediately after stimulating of MDA-MB-231 with CCL21 (50nM). (C), Dose response analysis showing changes in the maximal change in intracellular Ca2+ concentration measured in MDA-MB 231 following stimulation with either WT or Mutant CCL21. In vitro chemotaxis experiments: Measurement of PBMC/MDA cell migration across cytokine-activated endothelial cell monolayer grown on filters (trans-endothelial) (F) or across filter (trans-filter) (D&E). Invasion across the membrane was assessed after 2 h for PBMC and 24 h for MDA-MB-231. N=4.

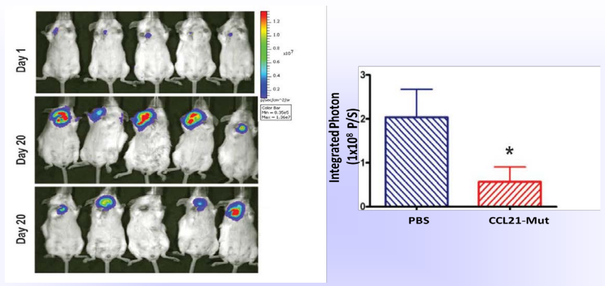

Figure 4: Determine the potential of mutant CCL21 for therapeutic blockade of the migration of Breast Cancer cells *in vivo*

Acknowledgment

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