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CCR5 upregulation in two subtypes of tumor associated circulating cells predict worse prognosis in metastatic breast cancer

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ABSTRACT

C-C chemokine receptor type 5 (CCR5) is a motility marker implicated in tumor progression, whose activation and endocytosis may identify highly aggressive breast cancer (BC) subtypes likely to spread via the circulatory system. We first studied the activation and endocytosis of CCR5 in response to its ligand RANTES in the model BC cell line MDA-MB-231. We then screened two types of circulating tumor-associated cells (TACs) with known clinical outcomes, 1) circulating tumor cells (CTCs) and 2) cancer-associated macrophage-like (CAMLs) cells to evaluate CCR5 upregulation in relation to disease progression in 54 metastatic breast cancer (mBC) patients.

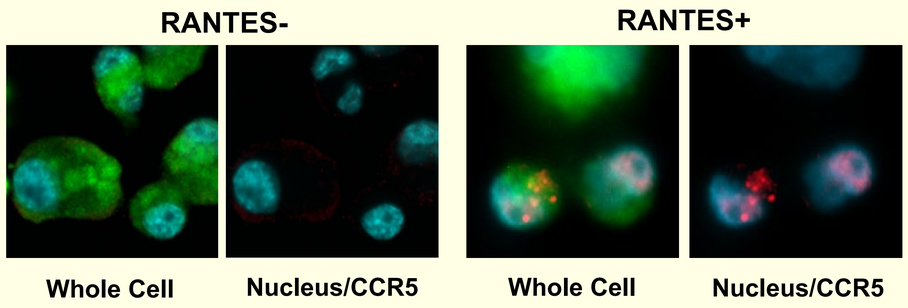

Figure 1. Cells without RANTES have no CCR5 expression (left panels), while cells with RANTES expressed CCR5 (red, right panels)

INTRODUCTION

The CCR5/RANTES axis is involved in regulating cancer cell migration and metastasis-promoting cell populations by helping cancer cells recruit and educate cells like monocytes to join the immunosuppressive tumor microenvironment (TME)[1,2]. CCR5 has been identified on CTCs in the blood system and CAMLs may be aiding in the transendothelial migration of CTCs into circulation[3,4]. We theorized that if similar patterns of CCR5 signaling can be found in model cancer cell lines and BC patients, then upregulation of CCR5 in patients may offer novel clinical applications.

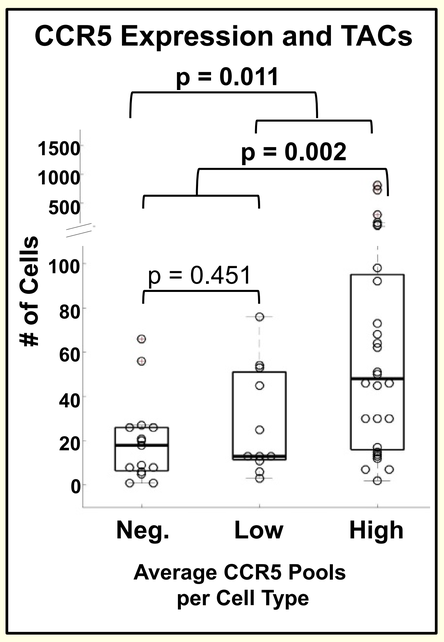

Figure 2. The correlation between CCR5 expression and the presence of all TAC populations in blood (n=54).

MATERIALS & METHODS

MB231 cells were used to visualize CCR5 activation after stimulation with RANTES. Anonymized peripheral blood samples from (n=54) mBC patients were obtained from Fox Chase Cancer Center and the University of Maryland in accordance with local IRB regulations and with written informed consent (4 patients did not have follow up survival available). TACs were isolated using CellSieve microfiltration system and stained for Cytokeratin, CD45, and CCR5. CCR5 expression signal was evaluated on TACs via fluorescence microscopy, and the number of CCR5 pools was manually enumerated.

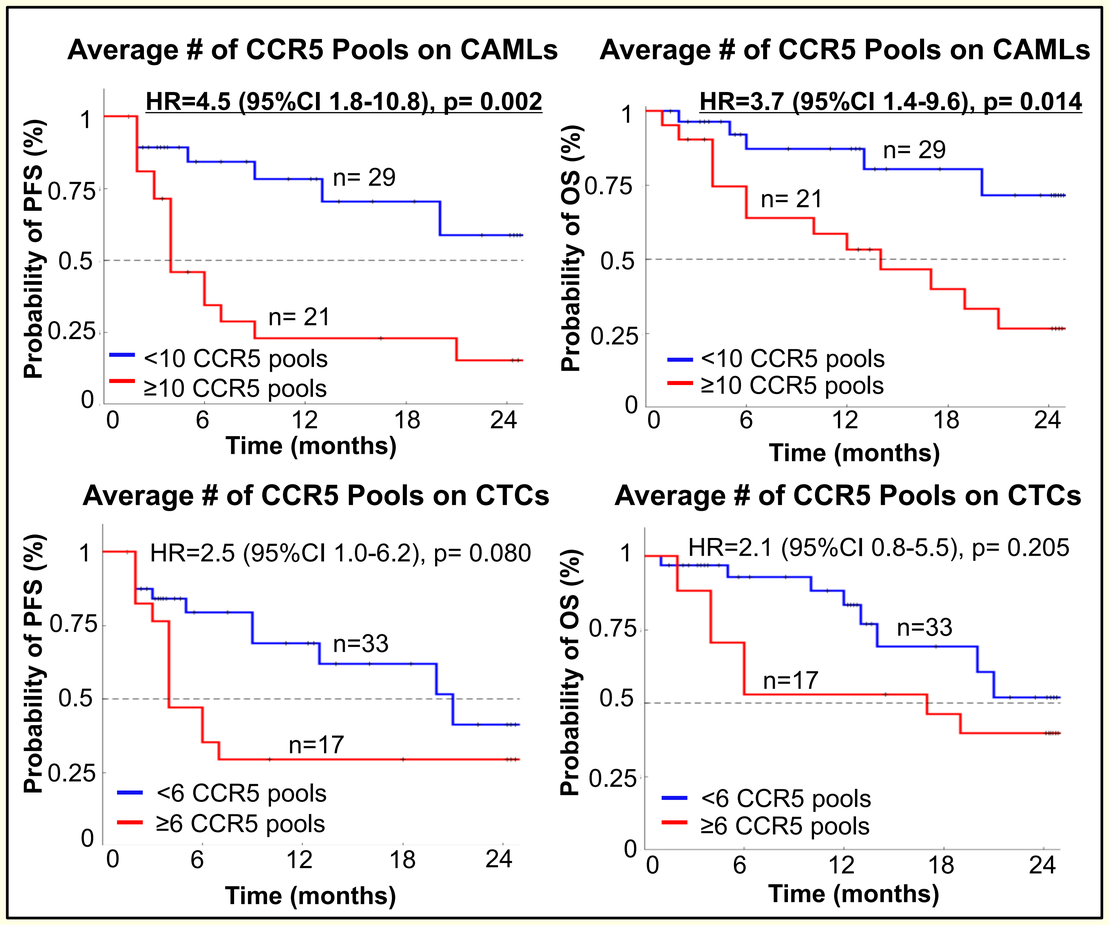

Figure 3. Presence of CCR5 on CAMLs and CTCs and their clinical significance. (Follow up was only available for n=50 patients)

RESULTS

- CCR5 appeared as ~1µm clusters, defined as "CCR5 pools", that were upregulated with the addition of RANTES (Figure 1).
- In mBC patients, 70% of patients (n=38) had CCR5+ CAMLs and 41% (n=22) had CCR5+ CTCs.
- Higher numbers of CCR5 pools (≥10 pools/cell) correlated to a 2-fold increase in TACs (Figure 2).
- An average of ≥10 CCR5 pools/cell on CAMLs was a significant predictor of worse PFS and OS (Figure 3).
- An average of ≥6 CCR5 pools/cell on CTCs trended towards worse PFS and OS (Figure 3).

CONCLUSIONS

- We observed the activation and endocytosis of CCR5 pools.
- The observed CCR5 motility pathway appears to be conserved in migratory cells (i.e. CTCs & CAMLs) in mBC patients.
- High CCR5 in CAMLs appears prognostic for worse clinical outcomes.
- High CCR5 in CTCs appears weakly associated with clinical outcomes.
- Finding CCR5 on TACs may indicate patients that would respond to anti-CCR5 therapies.

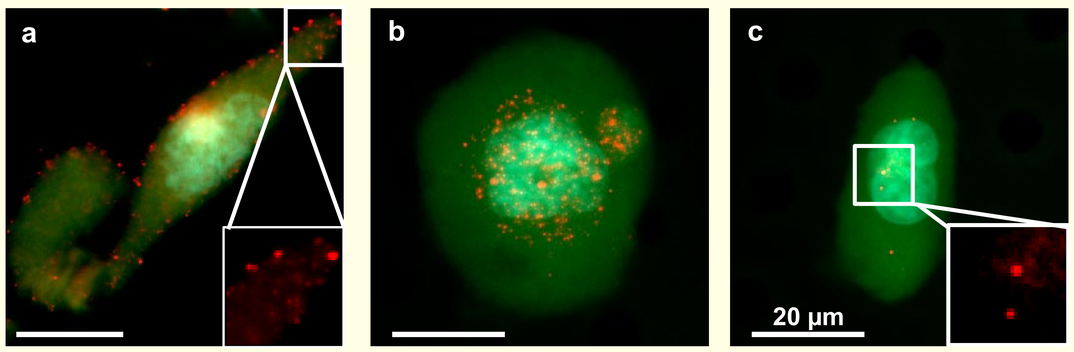

Figure 4. CCR5 expression in CAMLs isolated from mBC patients (Red=CCR5, Blue=nucleus, Green=Cytoplasm). (a) Externalized CCR5 signal. (b) internalized signal in the nucleus. (c) Low external and internal signal, with few CCR5 pools. Zoomed image (white boxes) magnifies CCR5 pools.

REFERENCES

[1] de Oliveira, C.E., et al. CC chemokine receptor 5: the interface of host immunity and cancer. *Dis Markers*, 126954 (2014).
[2] Aldinucci, D., et al. The CCL5/CCR5 Axis in Cancer Progression. *Cancers* 12 (2020).
[3] Cristofanilli, M. *Liquid Biopsies in Solid Tumors*, *Springer Publishing* (2018)
[4] Adams, D.L., *et al.* Circulating giant macrophages as a potential biomarker of solid tumors. *PNAS* 111, 3514-3519 (2014)

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