Metastatic Breast Cancer Cell Communication Within a Pro-Dormancy Bone Marrow Niche

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Introduction

Distant metastasis is a major cause of death in breast cancer with late relapse of disease occurring after years of tumour dormancy. The bone marrow (BM) has been suggested to serve as a protective environment for disseminated breast cancer cells (BCCs), however the precise molecular signals that circulating BCCs use to identify and adhere to BM vasculature is unknown. In this study, transcriptome analysis from publically available breast cancer data sets was used to identify correlations between late recurrence of disease and known molecular signals associated with BM homing and retention. Analysis of tumour cells and tumour-associated stroma identified a positive correlation between CXCR4/SDF-1 and E-selectin ligand transcript expression and late recurrence. *In vivo* experimentation using intravital confocal microscopy revealed that E-selectin and SDF-1 possess specific roles in regulating homing, retention and proliferation of BCCs. Use of the specific E-Selectin inhibitor, GMI-1271, significantly inhibited BCC homing to the BM. Inhibition of the SDF-1 / CXCR4 axis (using AMD-3100 or anti-CXCR4 neutralizing antibodies) had no inhibitory effect on homing, but could induce mobilization of BCCS out of the BM and into circulation. Proliferation of BCCs was also reserved to lateral BM regions, while dormant BCCs were localized to sinusoidal regions where SDF-1 and E-selectin are highly expressed.

Results

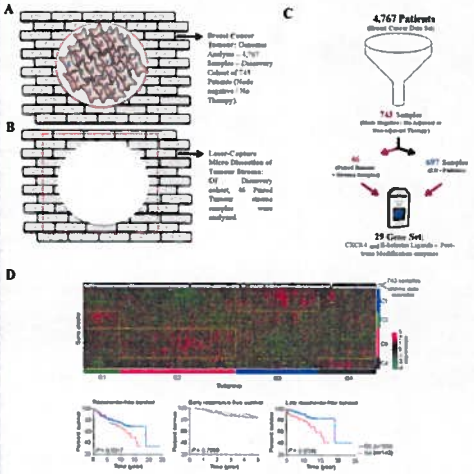


Fig 1: - Genomic Analysis of Breast Cancer Patient Data Sets: (A - B) Experimental design of breast cancer genomic analysis. 25 publically available data sets (4,767 breast cancer patients) were used (NCBI GEO). Analysis was performed on a discovery set of 743 node-negative / no therapy samples. Four subgroups with distinct genomic expression profiles (G1 – G4) and prognostic characteristics were identified (Subgroups of interest: G3 – "good prognosis" – G4 – "late recurrence"). Comparison of subgroups G3 and G4 is graphed, depicting differences in total, early (<5 years post diagnosis) and late (>5 years) recurrence-free survival. Data published, Cheng et al, *Breast Cancer Research* 2014, 16:407.

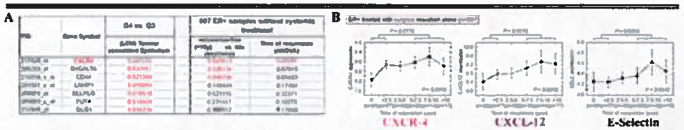


Fig 2: - CXCR4 / E-Selectin Ligands are Upregulated in "Late Recurrence" (G4) Data set: (A) Statistical breakdown of genomic analysis. Of the 743 node negative / no therapy patient samples identified, 46 samples contained laser capture microdissected (LCM) tumour epithelium. Expression of 29 genes representing CXCR4, E-selectin ligands, and enzymes critical for post-translational processing of E-selectin ligands in these tumor epithelium samples were analyzed, with 7 significantly elevated in G4 "late recurrence" patients. These 7 genes were analyzed in the ER+ subset (697 of 743 patients), with 3 of these 7 genes (including CXCR4) significantly upregulated in primary tumors with late recurrence (recurred >5y) (G4), compared with samples without evidence of recurrence for over 15 years (G3). (B) CXCR4, CXCL12 and E-Selectin transcript expression in the ER+ cohort was shown to be linearly correlated with time of recurrence.

Fig 3: - Intravital Confocal Microscopy and Video-rate Imaging of the Calvarial Bone Marrow: (A) *In-vivo* confocal microscopy imaging of the calvarium of the mouse is used to achieve single-cell resolution of BCC bone marrow homing. This method allows for measurement of the relative number, localization and micro-environmental interactions of bone marrow homed BCCs. BCCs are visualized using fluorescent transgenes or labelled with Fluorescent lipophilic dyes. Video-rate imaging and quantification of circulating cells or cells mobilized out of the bone marrow is performed using this technique. (B) Lipophilic dye content denotes dormancy. Dye is depleted after successive rounds of cell division. Proliferative cells are not visible in dye channel.

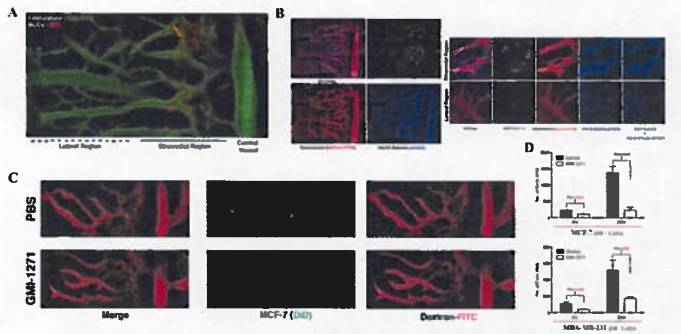


Fig 4: - Sinusoidal Vasculature E-Selectin Regulates BCC Homing and BM Entry: (A) Mouse engrafted with dye labelled (DiD) BCCs and homing of cells to the BM investigated via calvarial imaging +20hr post engraftment. BCCS predominantly home to sinusoidal regions of the vasculature. (B) Mouse engrafted with BCCs and homing imaged +2hr post engraftment. E-selectin was visualized via administration of AF-647 conjugated E-Selectin Ab (1hr prior to imaging). BCCs home to sinusoidal regions of E-selectin expression. (C & D) E-selectin regulates BM homing of both ER+ and ER- BCCs. Administration of specific E-selectin inhibitor, GMI-1271, significantly inhibits homing of BCCs. (C) – Representative image of calvarium for MCF-7 cell homing +/- GMI-1271 at +20hr post engraftment. (D) – Graph of No. of cells in BM +/- GMI-1271 at +2hr and +20hr post engraftment; MCF-7 and MDA-MB-231.

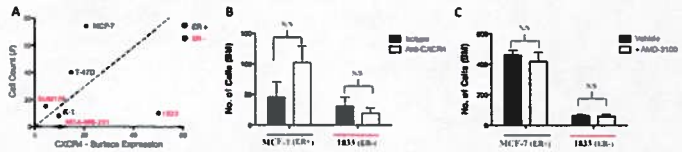


Fig 5: - CXCR-4 / SDF-1 Does Not Regulate BCC Homing: (A) Cell surface expression of CXCR4 on BCCs (ER+ & ER-) does not correlate with homing capacity. (B) Homing experiment using MCF-7 (ER+) and bone tropic 1833 (ER-) cells +/- incubation of anti-CXCR4 neutralizing antibody. Counts performed 2hr post engraftment. (C) Homing experiment using MCF-7 (ER+) and 1833 (ER-) cells +/- CXCR4 / SDF-4 inhibitor, AMD-3100. Counts performed +20hr post engraftment.

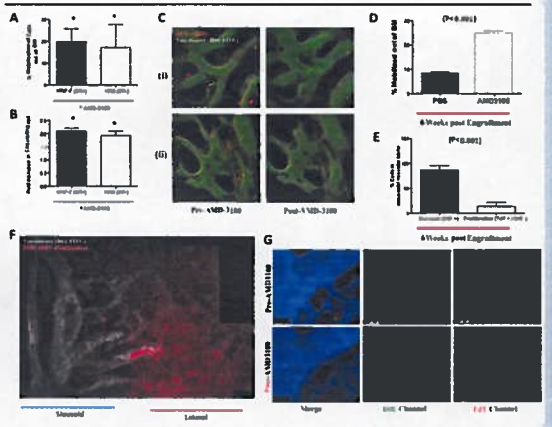
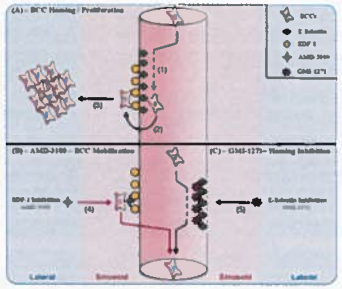


Fig-6: - CXCR4 Retains BCCs in a Pro-Dormancy Sinusoidal Niche: (A) Homing of BCCs quantified +20hr post engraftment, before and after (+4hr) treatment with AMD-3100. Reduction in No. of cells present in the marrow (% Mobilization) is graphed. (B) No. of DiD+ BCCs circulating in the vasculature pre and post AMD-3100 treatment was quantified. Graph depicts fold increase in No. of circulating cells. (C) Representative images [i & ii] of 1833 mobilization after treatment with AMD-3100. (D) AMD-3100 treatment mobilized cells out of the BM in mice with established disease. Mice were engrafted with 1833-TdT + DiD. AMD-3100 treatment performed 6 weeks after engraftment. (E) Dormant cells (lipophilic dye retained) and proliferative cells (TdT +) reside in separate anatomical regions. Dormant cells are predominant in the sinusoidal niche. (F) Mouse engrafted with 1833-TdT + DiD. Merged image of calvarial bone marrow performed 6 weeks after engraftment. Proliferative, TdT + 1833 cells are shown to proliferate predominantly in the lateral regions. (G) AMD-3100 mobilization experiment in mouse engrafted with 1833-TdT + DiR. AMD-3100 treatment and imaging performed approx. 6 weeks post engraftment. AMD-3100 mobilizes dormant, DiR + cells present in the sinusoidal niche. Representative images, pre and post (+4hr) AMD-3100 treatment are shown.

Model



Model of BCC Metastasis. (A) Circulating BCCs home to BM Vasculature via interaction with E-Selectin (1), while CXCR4 / SDF-1 interaction facilitates retention (2). Dormant BCCs remain in SDF-1 / E-selectin rich sinusoids, with proliferation of BCCS in lateral regions (3).
(B) Inhibition of the CXCR4 / SDF-1 axis (AMD-3100) can facilitate mobilisation of dormant BCCs.
(C) Inhibition of E-selectin (GMI-1271) can inhibit homing of BCCs to the bone marrow.

Discussion

This study reveals the specific roles both SDF-1 and E-selectin signaling play in trafficking of metastatic BCCs in and out of the BM. Employing available small molecule inhibitors of either pathway, GMI-1271 & AMD-3100, is shown to prevent homing of cells and promote mobilization of cells out of the BM respectively, and may represent viable therapeutics for the treatment and prevention of late recurrent disease in breast cancer.