Characterizing CCR7 Gene Amplification and Protein Expression in Inflammatory and non-inflammatory Breast Cancer

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Presentation Number: PS4-01-10

Introduction

- Inflammatory breast cancer (IBC) is an aggressive breast cancer subtype with a propensity for lymphovascular invasion and rapid metastatic spread. C-C chemokine receptor type 7 (CCR7), expressed on several types of immune cells, including B cells, mature dendritic cells (DCs), and various T cell subsets like naive, regulatory central memory T cells and aberrantly expressed in multiple cancers, including IBC. CCR7 interacts with its specific ligands, C-C motif chemokine ligand, CCL19 and CCL21.
- **Normal role**: Immune cell trafficking (T cells, dendritic cells) via CCL19/CCL21 → lymph nodes
- **Role in cancer**: Guides tumor cells into lymphatics, enhances invasion → Metastasis.
- We hypothesize that CCR7 mediates tumor cell migration to lymphatics in aggressive breast cancers.
- We characterized CCR7 gene amplification, protein expression, and cellular distribution in IBC versus non-IBC to evaluate its potential as a therapeutic target.

Methods and Materials

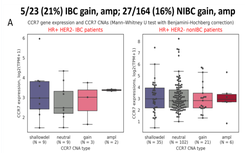
- CCR7 was assessed in clinically obtained genomic data by BostonGene in HR+ HER2-, HER2 and TNBC, versus subtype-matched non-IBC controls.
- IBC and non-IBC cell lines were subjected to immunoblotting for CCR7 expression and selected tumor cells and stromal cell lines were analyzed for ligand (CCL19 and CCL21) secretion (macrophages, dendritic cells, T cells, and HUVECs) by ELISA.
- Evaluation of CCR7 protein localization using sub cellular fraction immunoblotting and immunofluorescence, was performed with additional testing of a CCR7-blocking antibody.
- Multiplex immunofluorescence (mIF) was performed on BCM PDX sections representing different CCR7 amplification and expression groups.
- Functional studies included proliferation assay with CCR7 antagonist, Navarixin, ligand-stimulated migration assay using CCL19/21, and generation of CCR7 CRISPR knockdown clones.

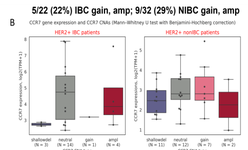
Results

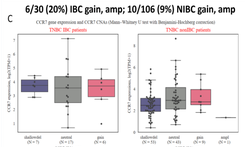
CCR7 gene amplification and expression in IBC and nonIBC patients

Samples selection

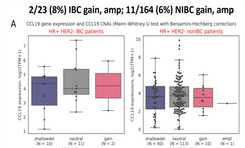
	HR+ HER2- Breast Cancer	HER2+ (HR+ & HR-) Breast Cancer	HR- HER2- (TNBC) Breast Cancer	Total
Inflammatory Breast Cancer (IBC)	23 samples	22 samples	30 samples	85 samples
NOT Inflammatory Breast Cancer (nonIBC)	164 samples	32 samples	106 samples	312 samples
Total	187 samples	54 samples	136 samples	377 samples

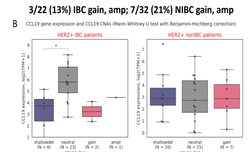


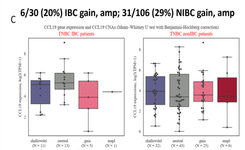




CCR7 genomic gains are found in ~ 20% across subtypes. IBC > nonIBC tumors for both Luminal (HR+) and TNBC subtypes .

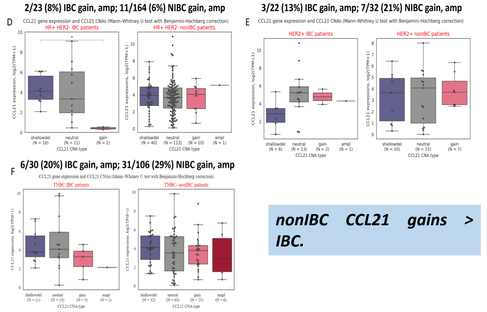






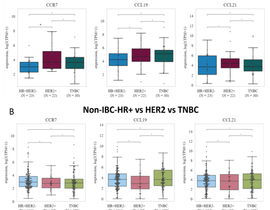
NonIBC CCL19 gains > IBC gains, specifically in HER2+ (21% NIBC vs. 13% IBC) and TNBC (29% NIBC vs. 20% IBC) subtypes.

Results



nonIBC CCL21 gains > IBC.

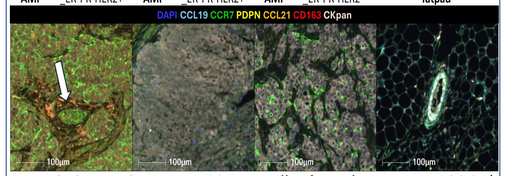
Gene Expression Comparison between IBC and Non-IBC



Across subtypes, CCR7 expression is higher in HER2+ ER- in IBC, unchanged in nonIBC.

Spatial Distribution of CCR7, CCL19/21 and Stroma Markers in Non-IBC PDX Confirms Expression and Protein Do Not Correlate

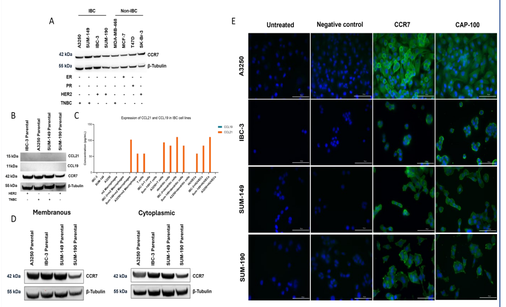
3104- CCR7 EXP^{HIGH} AMP^{GAIN} ER-PR-HER2+	3807- CCR7 EXP^{HIGH} AMP^{LOSS} ER-PR-HER2+	15029- CCR7 EXP^{LOW} AMP^{LOSS} ER-PR-HER2-	Hu- Normal mammary fatpad



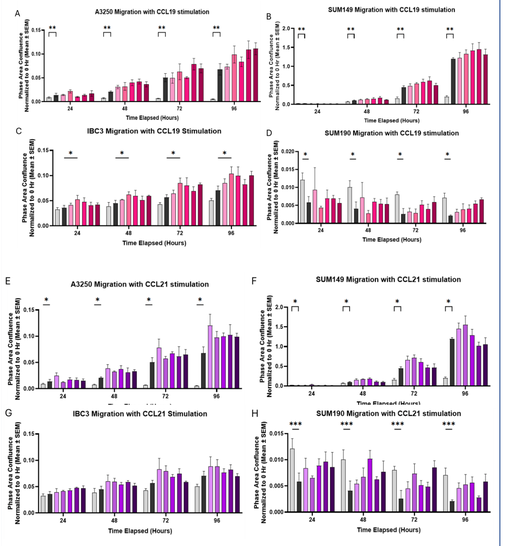
PDX 3104 – Common **CCR7+** cells (membranous positivity) including emboli (white arrow). **CCL21+** cells surrounding emboli.
PDX 3807 – Some nonspecific binding present in the connective tissue
PDX 15029 – Occasional tumor cells also show membranous positivity.

Results

CCR7 Expression and Localization in IBC Cell Lines

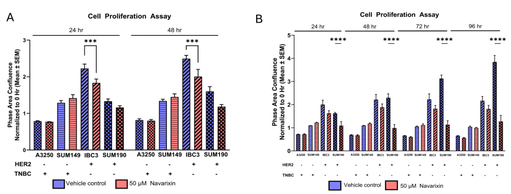


CCL19/21 ligand stimulation promotes CCR7-mediated migration in A3250 and SUM149 (TNBC)

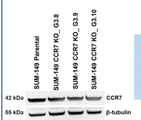


Results

Pharmacologic inhibition of CCR7 reproducibly decreased cell proliferation in IBC3 and SUM190 (HER2+)



Generation of CCR7 CRISPR knockout clones for mechanistic validation



CCR7 protein band (42 kDa) is significantly reduced in the knockout (KO) clones (predominantly in one CCR7-KO clone) compared to the Wild-Type (WT).

Conclusions

CCR7 expression in breast cancer is regulated independently of genomic copy number and present in IBC and nonIBC
Membranous CCR7 varies across IBC models.
CCR7 ligands and antagonists demonstrate cell line specific functional effects on proliferation and migration in IBC.

Next steps

- CRISPR targeting is in progress to test function through genetic manipulation.
- We will assess CCR7 and ligand function in a novel microfluidic model of IBC to examine vascular intravasation.

References

1. **Gadde, Manasa et al. "In vitro vascularized tumor platform for modeling tumor-vasculature interactions of inflammatory breast cancer." Biotechnology and bioengineering vol. 117,11 (2020): 3572-3590.**
2. **Gadde, Manasa et al. "Influence of Macrophages on Vascular Invasion of Inflammatory Breast Cancer Emboli Measured Using an In Vitro Microfluidic Multi-Cellular Platform." Cancers vol. 15,19 4883. 8 Oct. 2023.**

Acknowledgement

The authors express their gratitude to the NIH for the financial support provided under Grant **1R01CA284102-01A1**, which made this research possible. We extend special thanks to our collaborators and core facilities listed below for their invaluable scientific contributions and partnership.

Cathy Rain Smith IBC Grant

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