Role of the Calcium-Sensing Receptor (CaSR) in Invasive Lobular Breast Carcinoma Metastasis to the Ovary

Ye Qin, Laura Savaria, Ahmed Mohammed Basudan, Osama Shah, Zheqi Li, Tiantong Liu, Nilgun Tasdemir, Lan Coffman, Esther Elia, Jennifer M. Atkinson, Peter Lucas, Adriana V. Lee, Steffi Oesterreich

UPMC Hillman Cancer Center, Magee Women’s Research Institute, Pittsburgh, PA, Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA, Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, Integrative Systems Biology Program, University of Pittsburgh, Pittsburgh PA; School of Medicine, Tsinghua University, Beijing, China; Department of Hematology & Oncology, University of Pittsburgh, Pittsburgh, PA; Department of Pathology, Magee-Womens Hospital, Pittsburgh, PA; Department of Pathology and NSABP, Pittsburgh, PA

Abstract

Background: ILC accounts for about 10 to 15% of all invasive breast carcinoma, affecting approx. 26,000-40,000 women in 2020 in the US alone. Patients with ILC have poorer long-term outcomes compared to patients when compared to Luminal A ILC. ILC does not only metastasize to the common site of ER+ breast cancer such as the bones, it is also three times more likely to spread to the ovaries, peritoneum, and gastrointestinal tracts compared to IDC, and these unique aspects of metastases remain poorly understood.

Methods: We performed next-generation sequencing on 11 paired primary and ovarian metastases and 13 orphan ovarian metastases. There was an enrichment of lobular histology, with 13 samples originating from ILC, 6 from IDC and 6 from mixed ILC/IDC. Phenotypical assays were performed in four ILC cells lines: MDA-MB-134, MDA-MB-340, BCaK4 and SUM44PE. We applied growth, trans-well and wound-scratch assays, and E2 responses experiments to understand role of ER in observed phenotypes. F-actin staining was performed to assess the effects on cell cytoskeleton. Finally, we analyzed downstream signaling such as MEK/ERK pathways using western blotting, and treatments with a series of small molecule inhibitors.

Results: Differential gene expression analysis identified the calcium-sensing receptor CaSR to be up-regulated in the ovarian metastases. Although the activated CaSR had no effect on growth, it promotes migration through activation of the MEK/ERK pathway associated with increased F-actin formation. Furthermore, we observed that activation of the estrogen receptor is required for the cell migratory phenotype induced by the CaSR.

Impact: Altogether, our study provides insight on the potential mechanism in which the upregulation of the CaSR supports ILC ovarian metastasis. We hope that these studies will not only deepen our understanding of ILC ovarian metastasis but will eventually lead to the development of more effective therapies and improve the outcome of patients with this understudied type of breast cancer.

Identification of CaSR

Using next-generation sequencing and well-established bioinformatical tools, we characterized a transcriptomic landscape that is specific to breast cancer metastases to the ovary. Then CaSR was identified as candidate gene.

Workflow of bioinformatical analysis

CaSR promote ILC migration

CaSR is a class C GPCR that is crucial for extracellular free ionized calcium homeostasis.

Activated CaSR improves the ILC cell migration in trans-well model

CaSR promotes F-actin formation

F-actin (green) immunofluorescence confocal microscopy and quantification

CaSR promotes MEK/ERK signaling

Activated CaSR could induce MEK/ERK signaling pathway, and MEK inhibitors blocked the migration promoted by CaSR

Estrogen response involved in CaSR-related migration

Ovarian metastatic samples

ICI and 4OHPT inhibited the migration promoted by activated CaSR

Estrogen Response is essential to ILC cell migration promoted by the activated CaSR

Summary

Our study provides insight on the potential mechanism in which the upregulation of the CaSR supports ILC ovarian metastasis, that might help to prevent and treat breast cancer in patients with ILC.

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