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

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Abstract

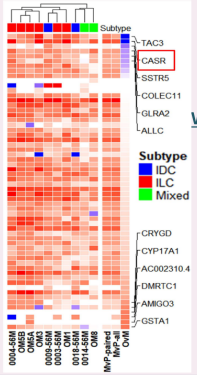
Background ILC accounts for about 10 to 15% of all invasive breast carcinoma, affecting approx. 26,000-40,000 women in 2020 in the US alone. Patients with ILC have poorer long-term outcomes when compared to patients with Luminal A IDC. ILC does not only metastasize to the common site of ER+ breast cancer 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-330, BCK4 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 upregulated 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.

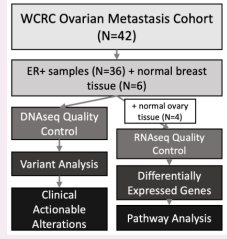
Impact Altogether, our study provides insight on the potential mechanism in which the upregulation of the CaSR supports ILC ovarian metastasis. We hope that these studies will not only deepen our understanding of ILC ovarian metastasis but will eventually lead to the development of more effective therapies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

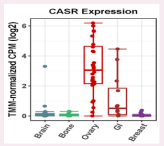
Identification of *CASR*

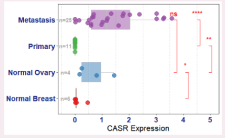


Using next-generation sequencing and well-established bio-informational tools, we characterized a transcriptomic landscape that is specific to breast cancer metastases to the ovary. Then *CASR* was identified as candidate gene.

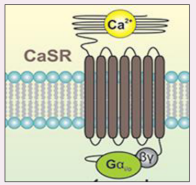
Workflow of bioinformatic analysis





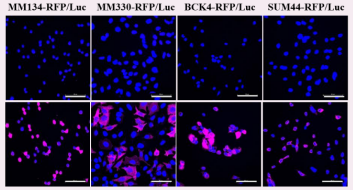


CaSR promote ILC migration

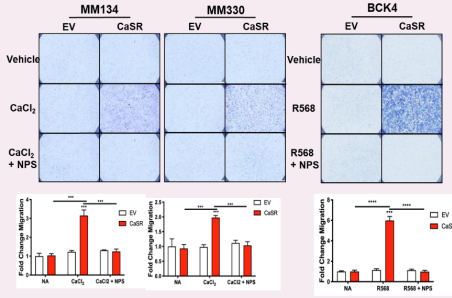


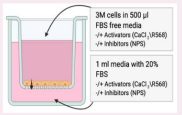
CaSR is a class C GPCR that is crucial for extracellular free ionized calcium homeostasis.

Over-expression of CASR in ILC cells

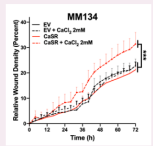


Activated CASR improves the ILC cell migration in trans-well model



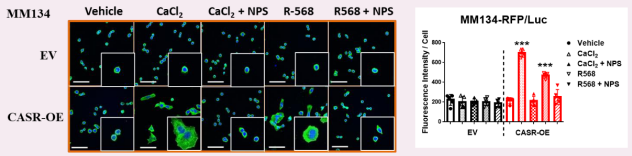


And in wound scratch



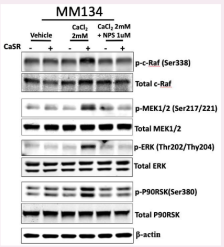
CaSR promote F-actin formation

F-actin (green) Immunofluorence confocal microscopy and quantification

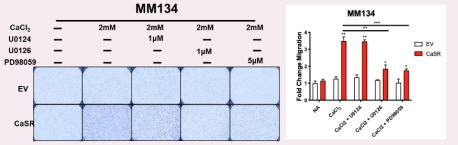


CaSR agoinst: $CaCl_2$ and R-568; CaSR antagoinst: NPS

CaSR triggers MEK/ERK signaling



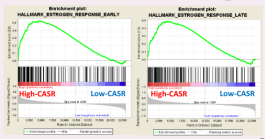
Activated CaSR could induce MEK/ERK signal pathway, and MEK inhibitors blocked the migration promoted by CaSR



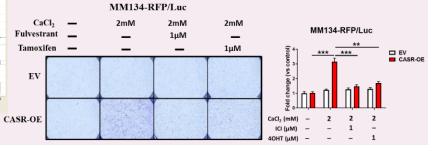
MEK inhibitors: U0126, PD98059; Negative control: U0124

Estrogen response involved in CaSR-related migration

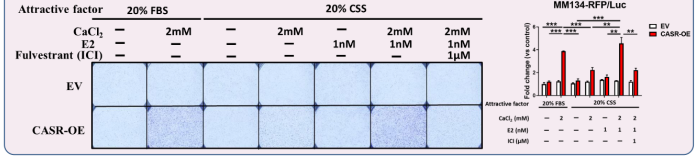
Ovarian metastatic samples



ICI and 4OHT 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.

Acknowledgement

Thanks to many anonymous patients donating most valuable tissues. We also appreciate the input from many breast cancer advocates on the project, especially members from LBCA.