Jagged-1 promotes breast cancer metastasis through the lymphatic system

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BACKGROUND & HYPOTHESIS

- 1 in 8 women will be diagnosed in their lifetimes. There is an urgent need to understand breast cancer (BC) metastasis due to low survival rate for distant spread.
- mRNA of Jagged1 (JAG1), a Notch ligand (Figure 1), correlates with decreased patient survival, node status, and metastatic relapse.
- While the lymphatic system is crucial for BC metastasis, little is known how the Notch pathway contributes to lymph node metastasis.

We hypothesize that JAG1 drives metastasis to the lymph nodes by inducing changes in the tumor lymphatic microenvironment via Notch and VEGF signaling.

RESULTS

I. JAG1 and Notch are significantly elevated in lymph node metastasis

![Figure 1. JAG1 (red) binds to Notch receptors, which drives the translocation of Notch-ICD into the nucleus.](Image)

II. Generation of JAG1\textsuperscript{LOW} vs JAG1\textsuperscript{HIGH} lymph node-invasive cell lines

![Figure 2. (A) Representative image of Jagged1 staining (green) and tumor mask (E-cadherin, red) of lymph node metastasis sample from a human BC tumor microarray used in quantification in B-C. (B) Primary tumors that have produced lymph node metastases contain more JAG1\textsuperscript{HIGH} cells. (C) More metastatic tumor cells express JAG1 protein than primary tumors. (B) JAG1 expression is higher in lymph node metastases when normalized to primary tumor in most patients. (E-F) A subset of MDA-MB-231 (MDA231) that metastasize to lymph nodes, MDA-MB-231-D3H2LN (LN) shows increased JAG1 expression. (B) MDA231-LN is enriched in Notch signaling pathways compared to MDA231. (*p=0.01 in 2F).

III. JAG1 promotes metastasis to the lymph nodes in tumor resection model

![Figure 4. (A) Orthotopic resection model in NCG (Charles River) mice allowed for metastasis tracking up to four weeks after removal of the primary tumor. (B) Primary tumors were removed at a consistent size. (C) After ~4 weeks, total lymph node burden increased in mice with JAG1 positive tumors (c), especially in mesenteric lymphatic tissue (D). (E-F) Representative axillary, subinguinal lymph node (E, arrows), and mesenteric (F) metastasis in mice with JAG1\textsuperscript{HIGH} tumor cells. (**p=0.01, ***p=0.001).

IV. JAG1 does not affect circulating tumor cell numbers after primary tumor is removed

![Figure 5. (A) Circulating tumor cells (CTC) detected by flow cytometry in blood from tumor-bearing mice. (B) JAG1 expression did not cause a significant difference in CTCs at two weeks post-resection or at ~4 week endpoint.](Image)

V. JAG1+ tumors increase peritumoral expression of lymphatic marker Lyve1

![Figure 6. (A) JAG1\textsuperscript{LOW} and JAG1\textsuperscript{HIGH} tumors were harvested at same size in MDA231-LN and 66cl4 models. (B) JAG1 does not affect the number of lymphatic cells (CD105\textsuperscript{+}/CD31\textsuperscript{+}/Lyve1\textsuperscript{+}, via flow cytometry) within the primary tumor in either model. (C) JAG1 expression increases the intensity of Lyve1 staining but not the number of lymphatic cells in the peritumoral mammary fatpad in mice with JAG1\textsuperscript{HIGH} 66cl4 tumors. (**p=0.001).](Image)

CONCLUSIONS

- JAG1 expression is significantly higher in cancer cells in the lymph nodes than in primary tumors in human BC patient samples.
- BC cells that home to the lymph nodes have higher JAG1 expression than other organ-tropic BC cell lines and parental line.
- In animal models, JAG1 significantly increases total lymph node metastasis.
- JAG1 expression does not impact CTC count after the primary tumor is removed.
- JAG1 expression in primary tumor increases Lyve1 intensity of surrounding lymphoendothelial cells.
- Ongoing investigation: determine the early points of lymphoendothelial invasion and changes to tumor microenvironment that may promote metastasis.

ACKNOWLEDGEMENTS

NCl NRSa, F30CA257269 (BG), DoD CDRP, BC170816 (JKK), UIH Research Histology and Imaging Core, UIH Cancer Center.