Contact me with questions!

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 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.

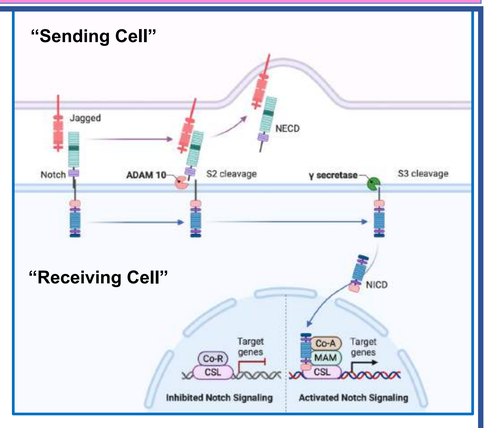


Figure 1. JAG1 (red) binds to Notch receptors, which drives the translocation of Notch-ICD into the nucleus.

RESULTS

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

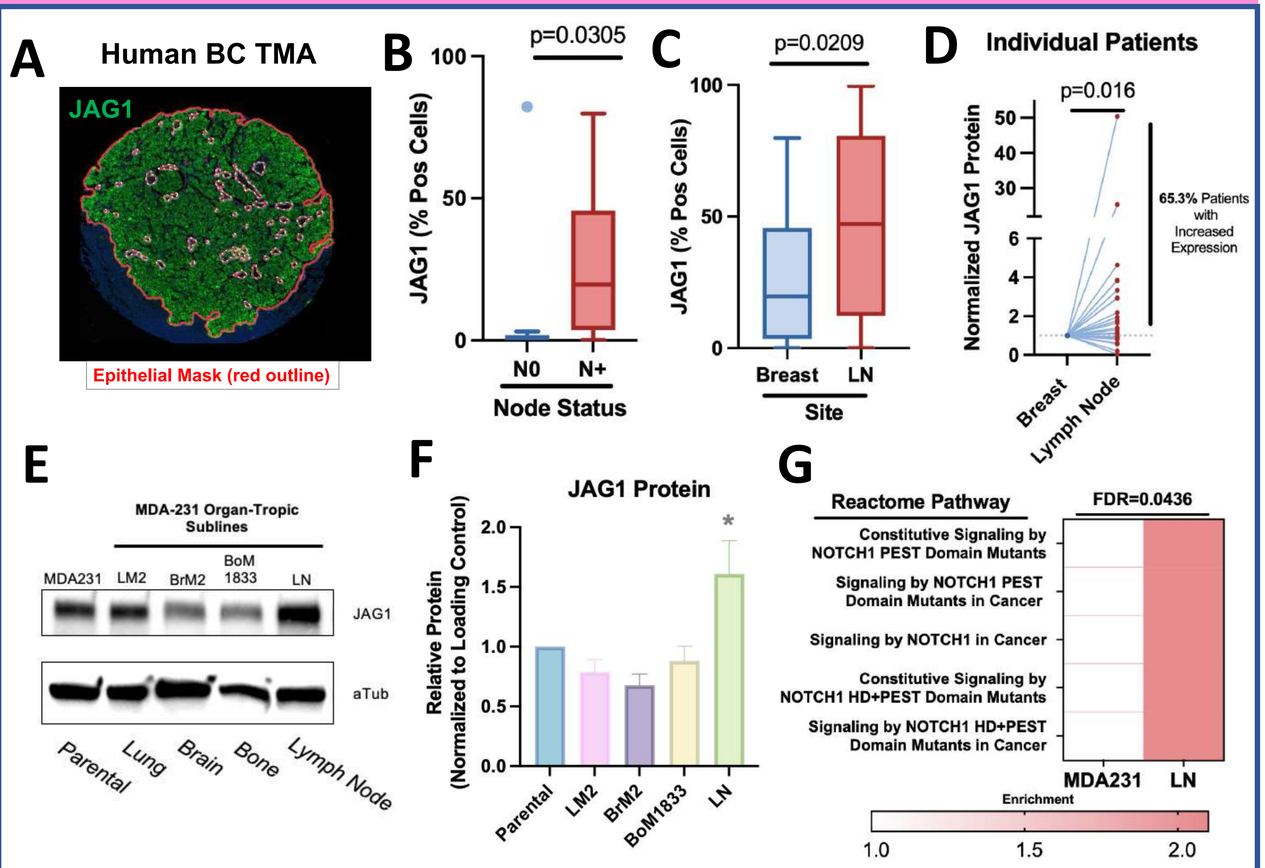


Figure 2. (A) Representative image of Jagged1 staining (green) and tumor mask (E-cadherin, red) of lymph node metastasis sample from a human BC tumor microarray used in quantification in B-D. **(B)** Primary tumors that have produced lymph node metastases contain more JAG^{POS} cells. **(C)** More metastatic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

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

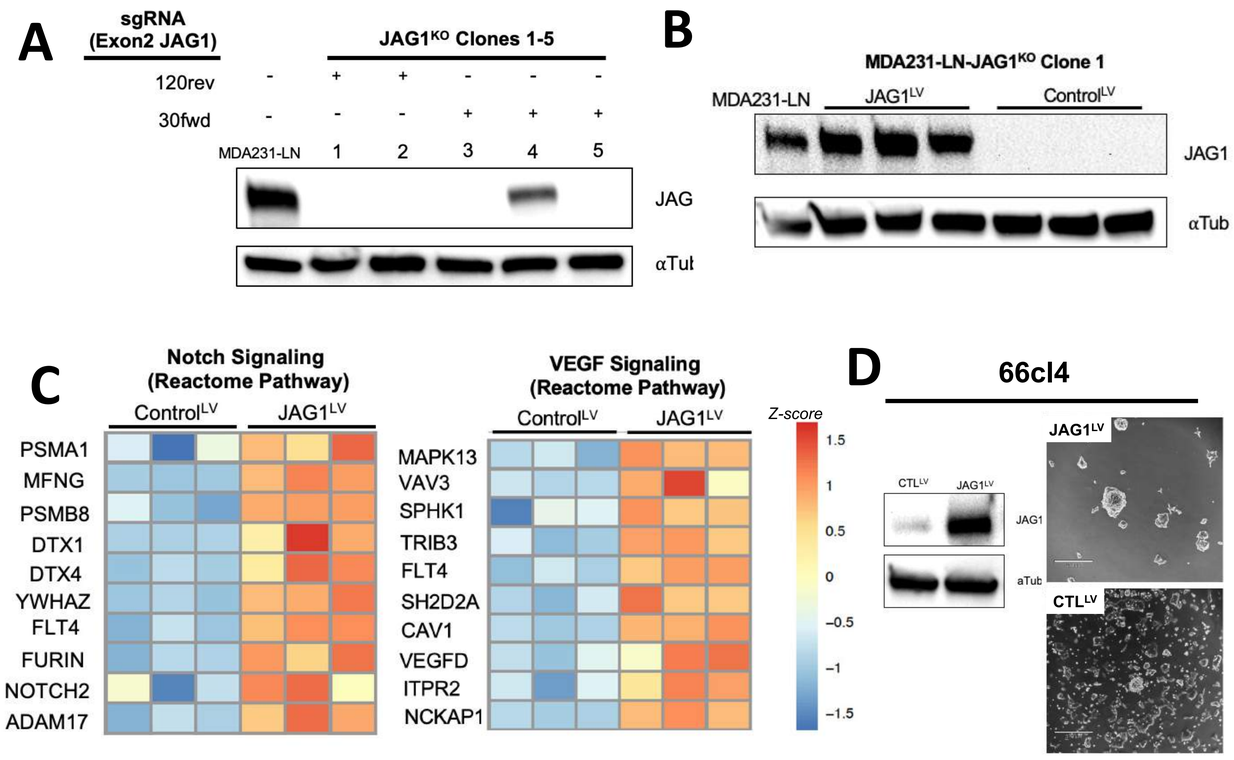
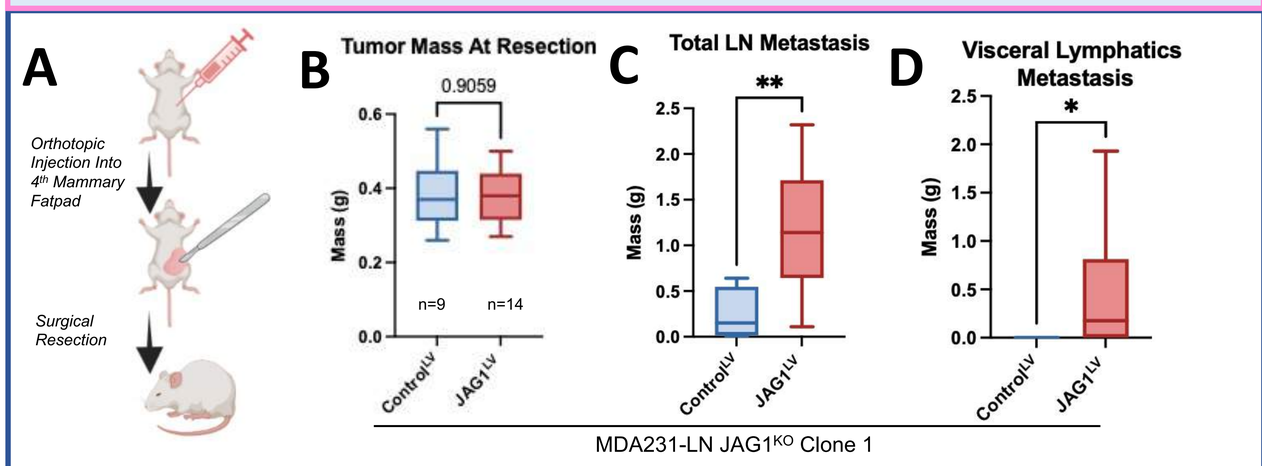


Figure 3. (A) To test JAG1 function in metastasis, five JAG1^{KO} sublines of MDA231-LN were generated using CRISPR/Cas9 to create homozygous frameshift mutations in exon 2 via distinct guide RNAs (120rev and 30fwd). Lines were confirmed by sequencing and Western blot. **(B)** To create matched JAG1 expressing lines, JAG1^{KO} Clone 1 was transduced with a JAG1 expression lentivirus (JAG1^{LV}). **(C)** Notch and VEGF signaling pathways are significantly enriched in JAG1^{LV} Clone 1 (heatmaps, top ten genes). **(D)** For confirmation, the cell line 4T1-66cl4, which has low endogenous Jag1 expression, was also transduced with JAG1 lentivirus, which caused a dramatic shift toward 3-D growth morphology.

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



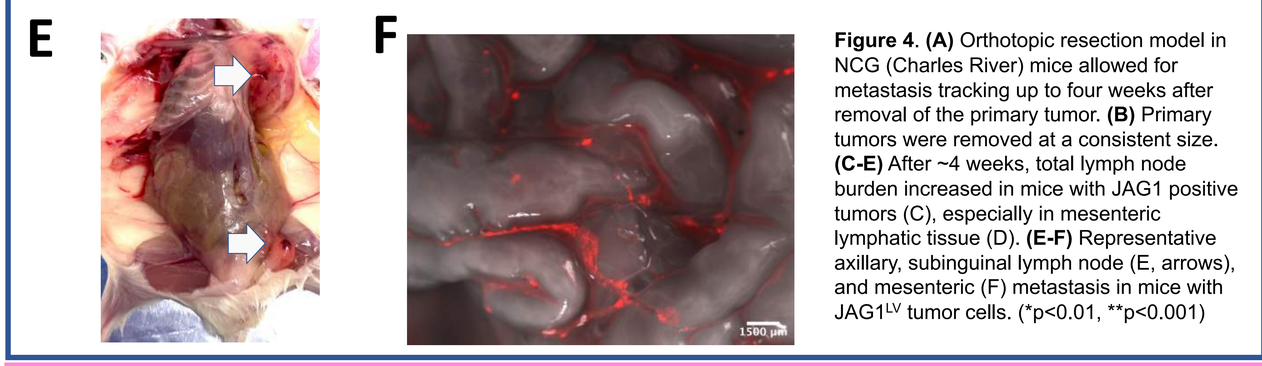


Figure 4. **(A)** Orthotopic resection model in NCG (Charles River) mice allowed for metastasis tracking up to four weeks after removal of the primary tumor. **(B)** Primary tumors were removed at a consistent size. **(C-E)** After ~4 weeks, total lymph node burden increased in mice with JAG1 positive 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^{LV} tumor cells. (*p<0.01, **p<0.001)

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

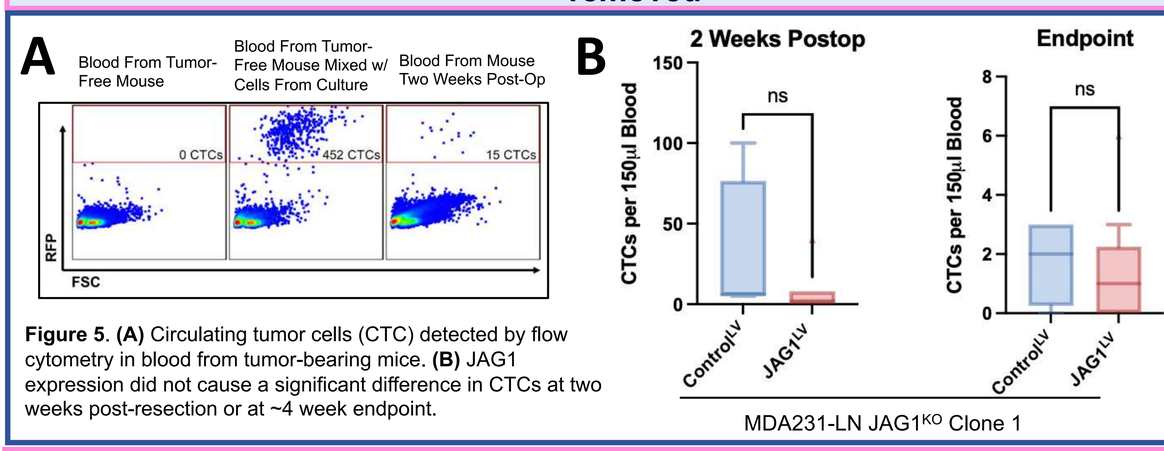


Figure 5. **(A)** Circulating tumor cells (CTC) detected by flow cytometry in blood from tumor-bearing mice. **(B)** JAG1 expression did not cause a significant difference in CTCs at two weeks post-resection or at ~4 week endpoint.

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

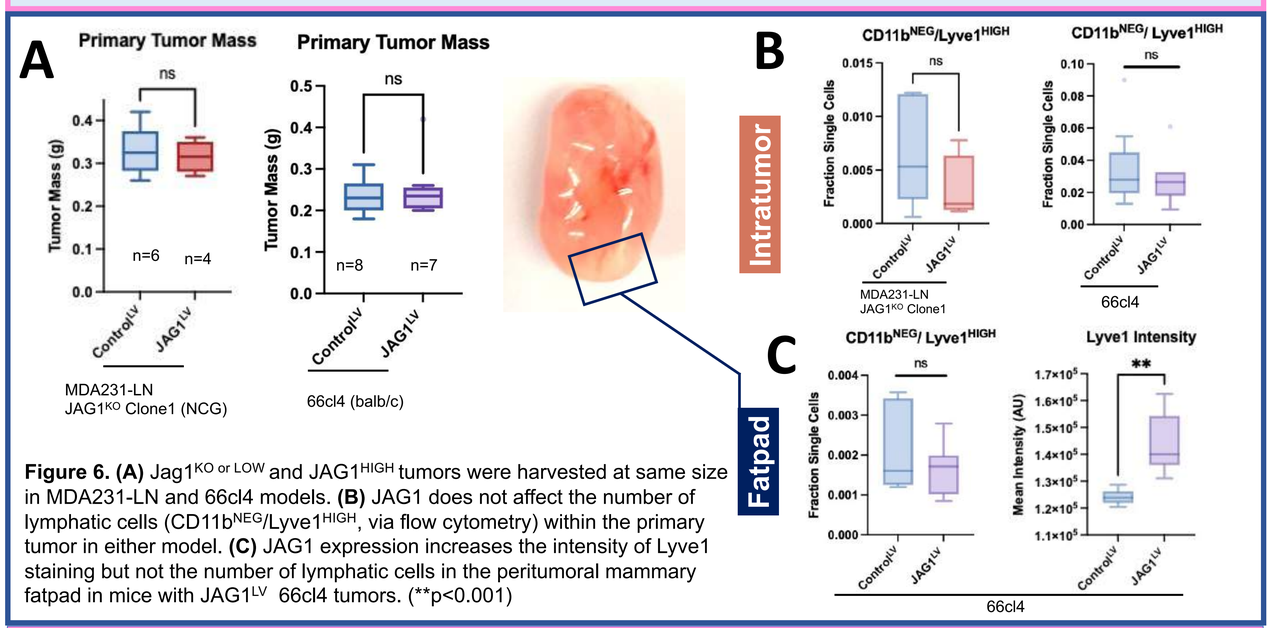
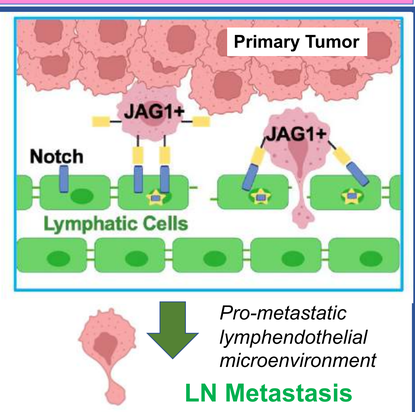


Figure 6. (A) Jag1$^{KO\ or\ LOW}$ and JAG1^{HIGH} tumors were harvested at same size in MDA231-LN and 66cl4 models. **(B)** JAG1 does not affect the number of lymphatic cells (CD11b^{NEG}/Lyve1^{HIGH}, via flow cytometry) within the primary tumor in either model. **(C)** JAG1 expression increases the intensity of Lyve1 staining but not the number of lymphatic cells in the peritumoral mammary fatpad in mice with JAG1^{LV} 66cl4 tumors. (**p<0.001)

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 lymphendothelial cells

- **Ongoing investigation:** determine the early points of lymphendothelial invasion and changes to tumor microenvironment that may promote metastasis



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