JAG1 Role in the Extravasation of Metastasized TNBC Tumor Cells

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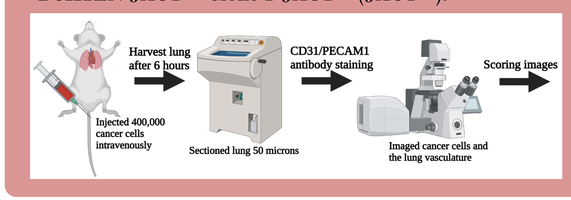
BACKGROUND & HYPOTHESIS

Breast cancer is the second leading cause of cancer death in women. Triple negative breast cancer (TNBC) is an aggressive subclass defined by its lack of hormonal receptors and HER2 amplification. Although TNBC only accounts for 15% of all invasive breast cancers, there are **limited therapeutic options** for patients with TNBC. Even though breast cancer patients have a favorable prognosis if their tumor is detected early, patients with TNBC are **prone to earlier recurrence and local/distant metastasis**. Consequently, patients with metastatic TNBC have **<15% relative 5-year survival rate**. The development of metastasis in TNBC is a complex and poorly understood process that includes multiple steps such as genetic and epigenetic alterations, angiogenesis, epithelial-to-mesenchymal transition (EMT), intravasation, and extravasation. Expression of JAGGED-1 (JAG1), a Notch ligand, correlates with metastatic status and poor survival in clinical data. However, the exact **mechanism in which JAG1 increases metastasis is unknown**.

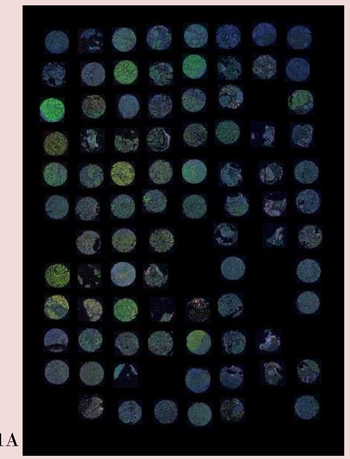
Hypothesis: We hypothesize that JAG1 increases TNBC metastasis by promoting cancer cell extravasation through the endothelial barrier.

METHODOLOGY

In order to test the hypothesis, we **generated JAG1-knockout cells** using CRISPR/Cas9 technologies and then **modeled the extravasation of these cells compared to JAG1-positive cells**. Specifically, we **interrogated lung capillary extravasation** after intravenous injection of TNBC cells. The lung was chosen due to the propensity of TNBC cells to invade the lung. In addition to KO, we **"rescued" JAG1 expression in knockout cells** using lentiviral (LV) mediated transduction. Group 1: Untreated MDA-MB -231-D3H2LN (D3-Parental); Group 2: D3H2LN JAG1^{KO} Clone 1 (RFP^{LV}); Group 3: D3H2LN JAG1^{KO} Clone 1-JAG1^{LV} (JAG1^{LV}).



RESULTS



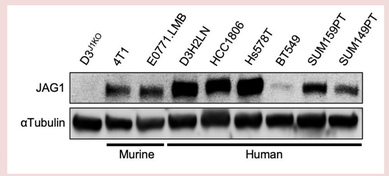
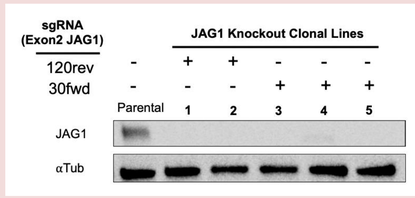


Figure 1. (A) Breast cancer tissue microarray (TMA) containing 12 cases of paired normal and primary, and 36 cases of primary and metastatic tumors (LNs) with JAG1 staining shown in green. Analysis of the TMA reveals that **(B)** JAG1 is higher in the primary tumor of patients with positive LNs and **(C)** metastatic LN samples have higher JAG1 compared to matched primary samples. **(D)** Western blot of human and murine TNBC lines probing for JAG1 expression. Subsequent results focuses mainly on MDA-MB-231-D3H2LN (D3H2LN), which is highly metastatic to LNs and lung.



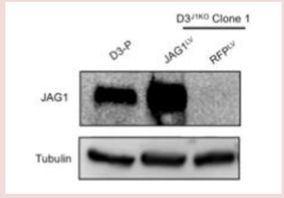
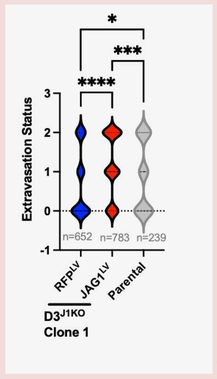
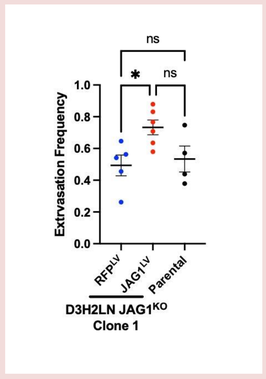


Figure 2. (A) Two separate guide RNAs (sgRNAs) targeting Exon2 of JAG1 were used to establish five distinct JAG1 knockout clonal lines from the parental D3H2LN line (D3-P), named D3^{J1KO} clonal lines 1-5. The sgRNA names refer to position on Exon2. Homozygous frameshift mutations were confirmed via next generation amplicon sequencing (Illumina miniSeq 2x150). **(B)** Rescue of JAG1 expression was performed in D3^{J1KO} Clone 1 using lentiviral (LV) methods.





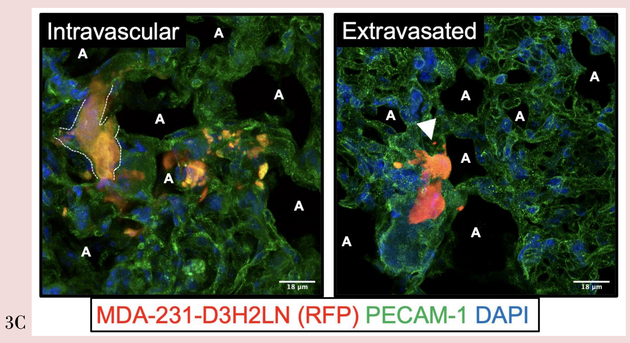


Figure 3. (A) Quantification imaging results shows JAG1 positive cells extravasate at a higher rate than knockout cells six hours after IV injection. n = each individual cell; 2 = extravasated; 1 = in-process; 0 = intravascular. **(B)** The extravsation frequency of cells is calculated by taking the number of extravasated and in-process and dividing it by the total. Each point constitutes the average extravasation per mouse. n = each mouse (fraction of in-process and extravasated cells). Error bars = SEM. * = p<0.05; ** = p<0.01; *** = p<0.001; **** = p<0.0001. **(C)** Representative 3D confocal-rendered reconstruction of intravascular and extravasated cells in the lung 6 hours after 400,000 D3-P, D3^{J1KO} C-1 RFP^{LV}, or D3^{J1KO} C-1 JAG1^{LV} cells were injected intravenously into NCG mice.

CONCLUSION & OUTLOOK

JAG1 presented in tumor cells acts as a signaler to permit and promote extravasation and metastasis of the cancer cells. JAG1 expression is higher in patient breast cancer cells that metastasize to LN compared to matched primary tumors. Moreover, primary tumor samples have higher JAG1 signals in aggressive clinical cases. JAG1 expression is robust in many aggressive murine and human TNBC cell lines. In addition, using CRISPR/Cas9 technologies, it was understood that JAG1 promotes extravasation of TNBC cells across the lung microvasculature in vivo. For **future experiments**, additional TNBC cell lines will be utilized. **Future** studies will track lung metastasis in orthotopic models. **In addition**, the long term effects of tumor derived JAG1 will be studied to understand the consequences of JAG1 mediated extravasation on metastatic burden and survival. **Future studies** will also assess proprietary JAG1-blocking agents.

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