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JAG1 Role in the Extravasation of Metastasized TNBC Tumor Cells

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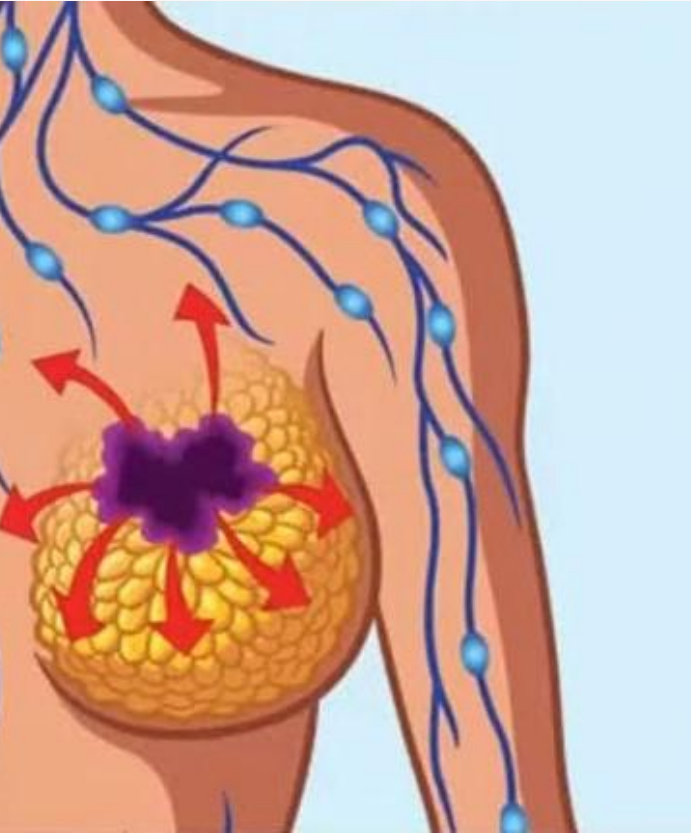
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01 BACKGROUND

Introduction & Hypothesis

INTRODUCTION



- Breast cancer → second leading cause of cancer death in women
- Triple negative breast cancer (TNBC) (Hossain et al., 2018)
 - Aggressive subclass defined by lack of hormonal receptors and HER2 amplification
 - Accounts for 15% of all invasive breast cancers
- Patients:
 - Have limited therapeutic options
 - Are prone to earlier recurrence and local/distant metastasis
 - <15% relative 5-year survival rate (Gordon et al., 2021)
- JAGGED-1 (JAG1) (Reedijk et al., 2005)
 - Notch ligand
 - Correlates with metastatic status and poor survival in clinical data
 - Exact mechanism in which JAG1 increases metastasis is unknown

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

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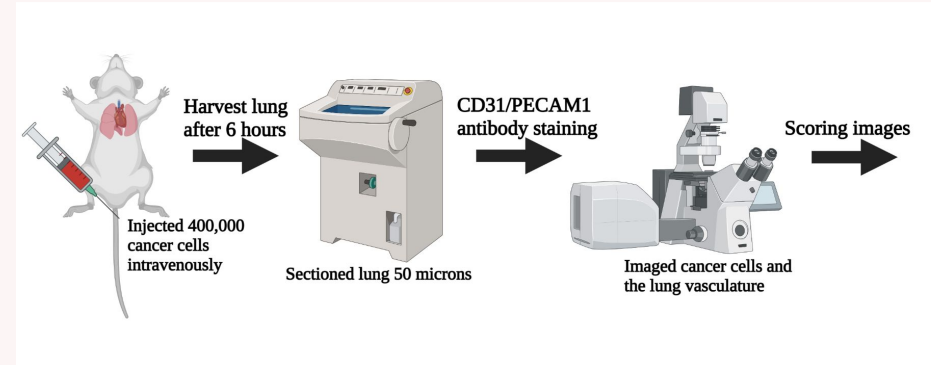
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Materials & Methods

METHODOLOGY

- Used TMA and western blot
- Generated JAG1-knockout cells using CRISPR/Cas9 technologies
- Modeled the extravasation of JAG1-knockout cells to JAG1-positive cells
- Interrogated lung capillary extravasation
 - Chosen due to propensity of TNBC cells to invade the lung
- “Rescued” JAG1 expression in knockout cells using lentiviral (LV) mediated transduction
- 3 Groups
 - 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})



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Figures & Captions

Figure 1: JAG1 May Promote Aggressive Metastatic Behavior in Breast Cancer

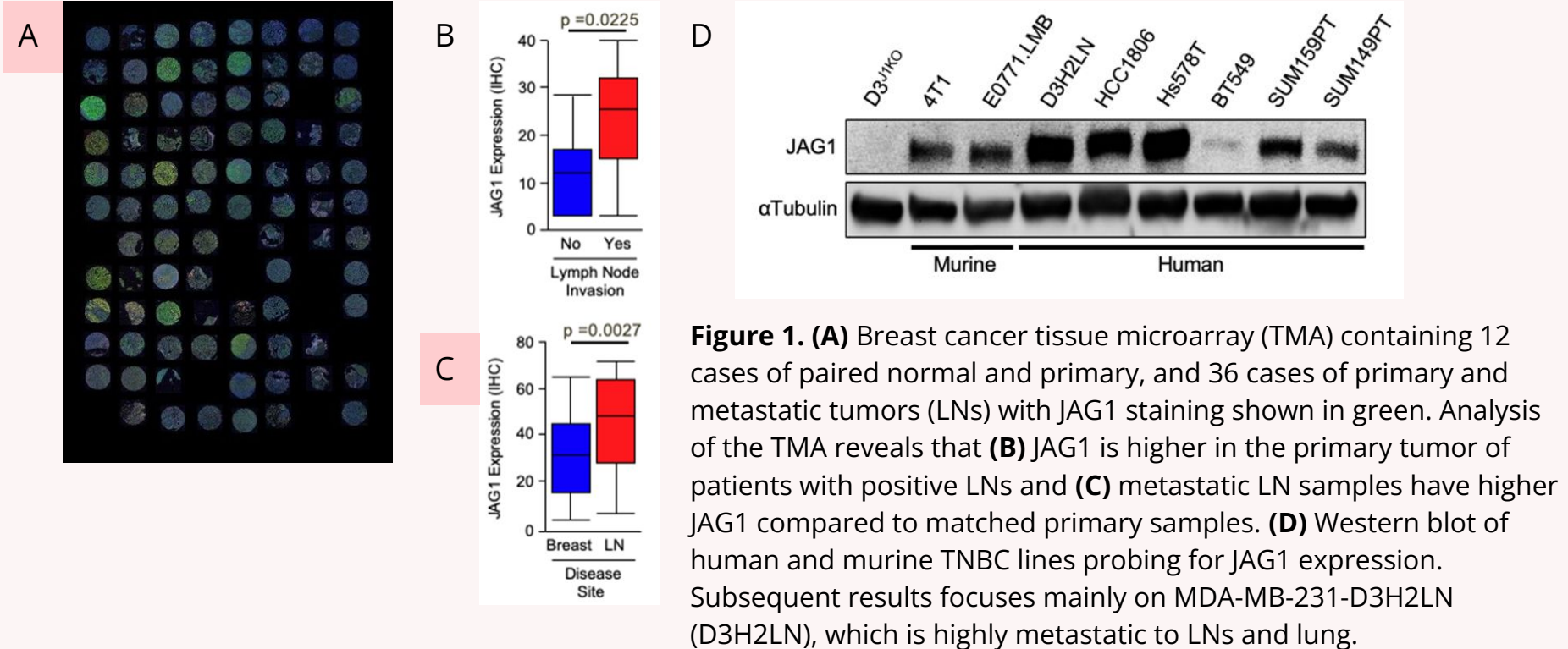
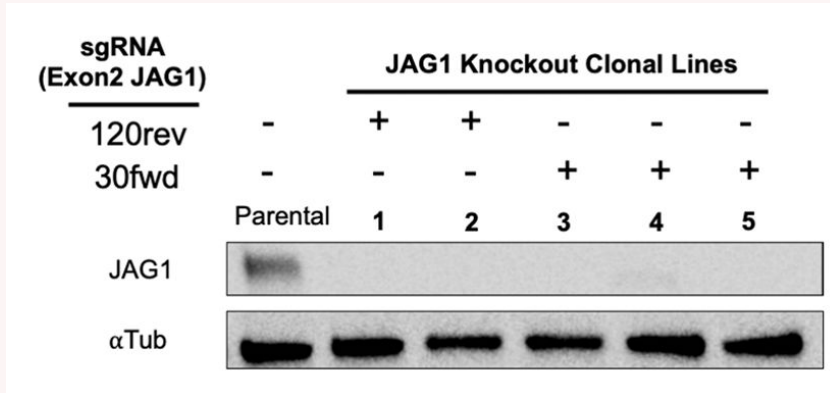


Figure 2: Generation of Five Knockout JAG1 Cell Lines Using CRISPR/Cas9

A



B

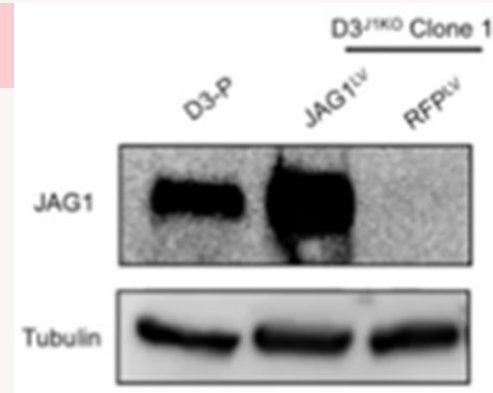


Figure 2. (A) Two separate guide RNAs (sgRNAs) targeting Exon2 of JAG1 were used to establish five distinct JAG1 knockout clonal lines from the parental D3H2LN line (D3-P), named D3^{1KO} clonal lines 1-5. The sgRNA names refer to position on Exon2. Homozygous frameshift mutations were confirmed via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

Figure 3: JAG1 Promotes TNBC Lung Metastasis and Extravasation

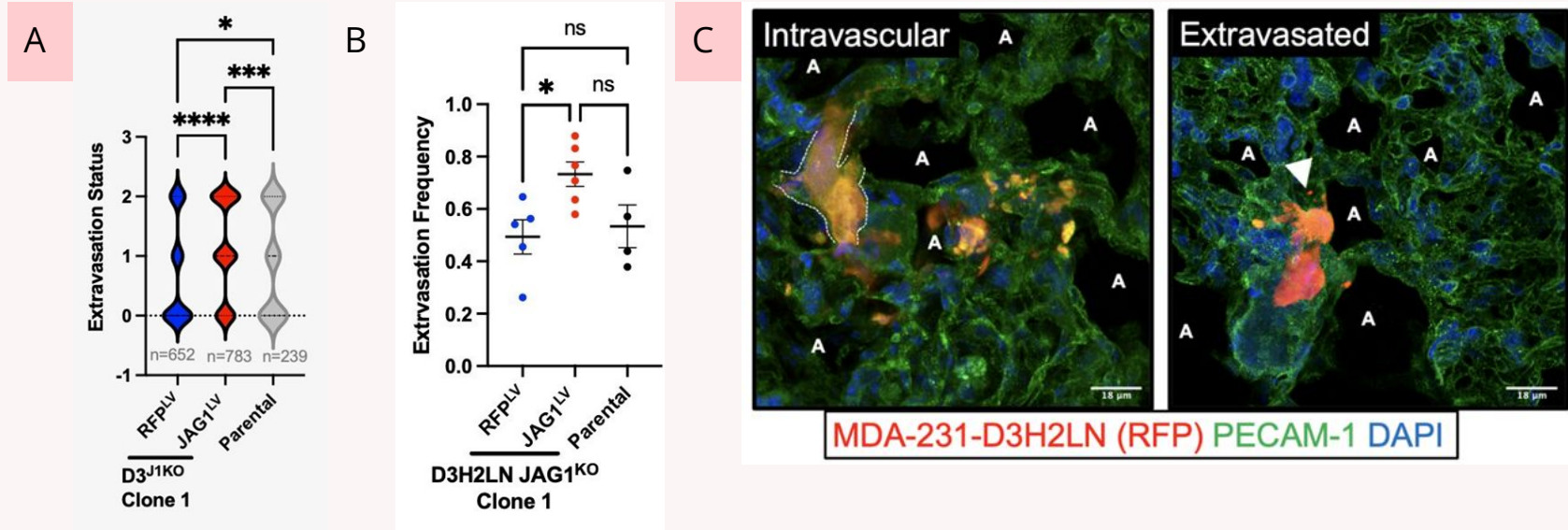


Figure 3. (A) Quantification imaging results shows JAG1 positive cells extravasate at a higher rate than knockout cells six hours after IV injection. n = each individual cell; 2 = extravasated; 1 = in-process; 0 = intravascular. **(B)** The extravasation frequency of cells is calculated by taking the 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.

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Outlook & References

CONCLUSIONS

- JAG1 acts as a signaler to permit and promote extravasation and metastasis of cancer cells
- JAG1 expression higher in patient breast cancer cells that metastasize to LN compared to matched primary tumors
- Primary tumor samples have higher JAG1 signals in aggressive clinical cases
- JAG1 promotes extravasation of TNBC Cells across lung microvasculature in vivo

OUTLOOK

- Future Experiments
 - Additional TNBC cell lines will be utilized
- Future Studies
 - Track lung metastasis in orthotopic models
 - Long term effects of tumor derived JAG1
 - Understand the consequences of JAG1 mediated extravasation on metastatic burden and survival
 - Assess proprietary JAG1-blocking agents

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