JAG1 Role in the Extravasation of Metastasized TNBC Tumor Cells

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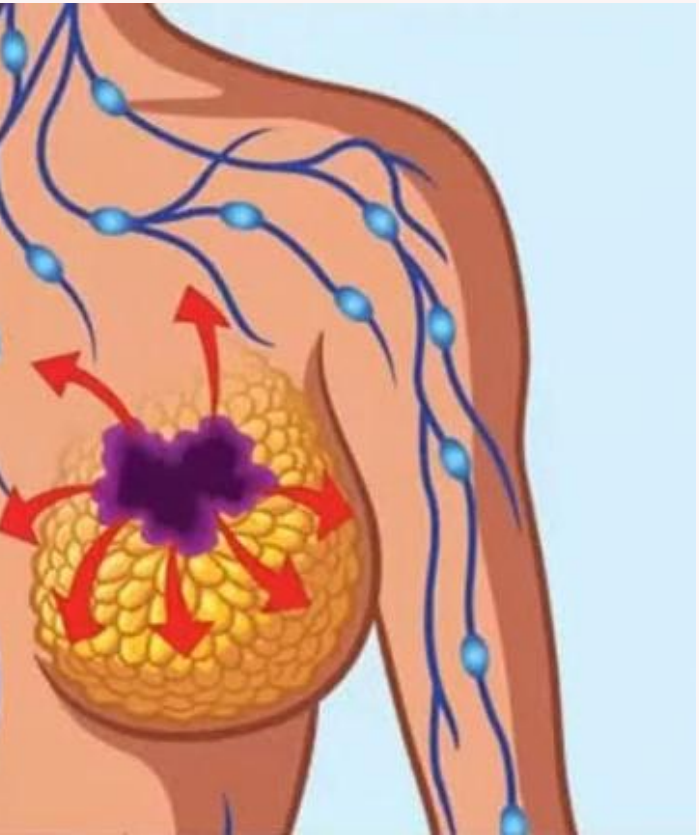
INTRODUCTION



- 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.

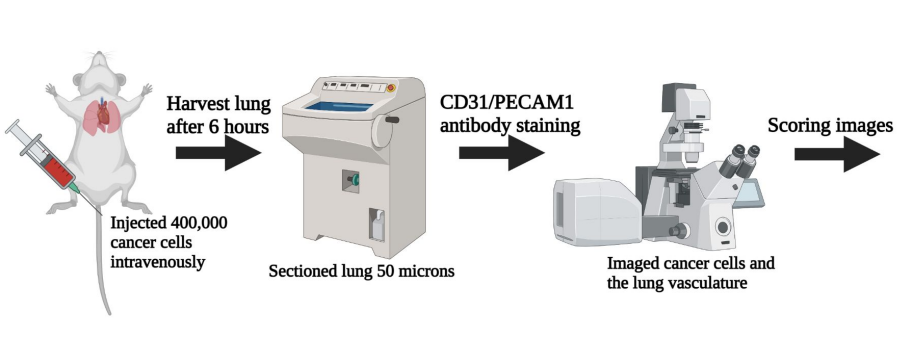
HYPOTHESIS

02 METHODOLOGY

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})

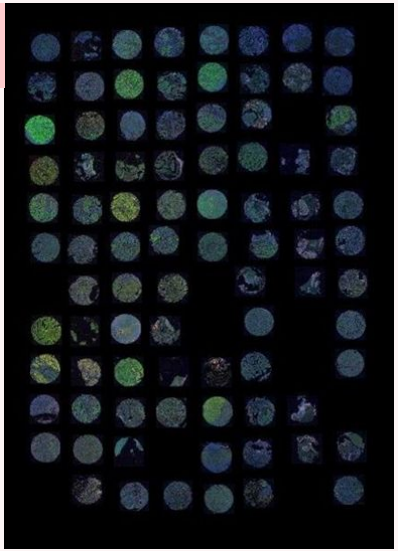


03
RESULTS

Figures & Captions

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

A



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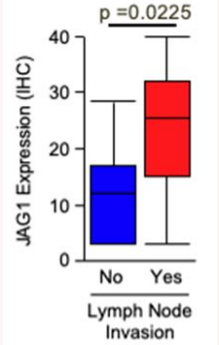

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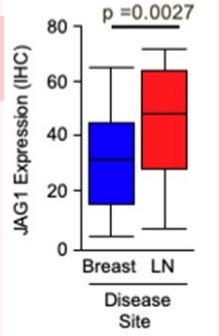

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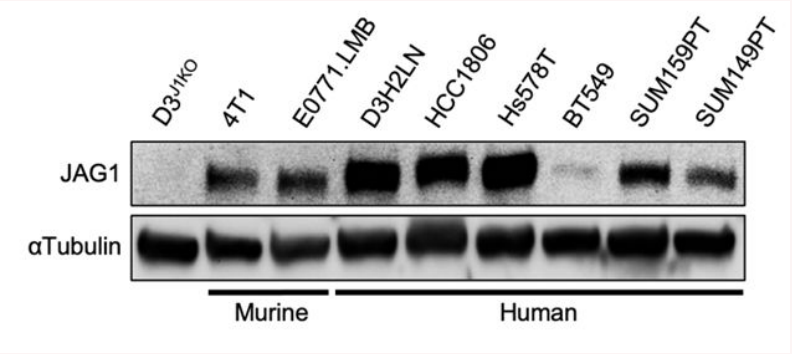
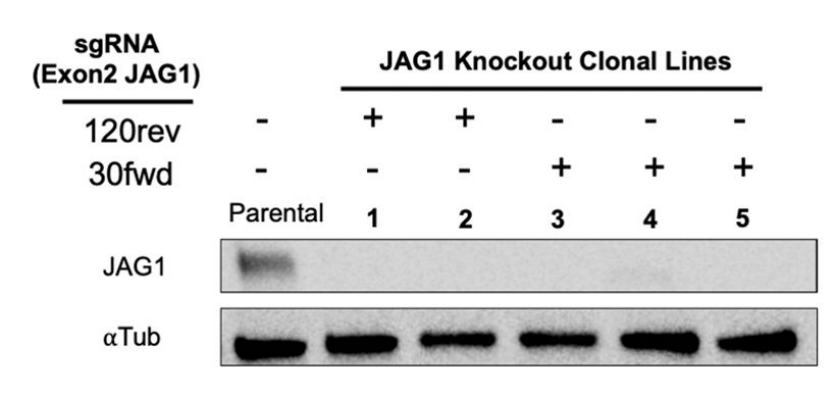


Figure 1. (A) Breast cancer tissue microarray (TMA) containing 12 cases of paired normal and primary, and 36 cases of primary and metastatic tumors (LNs) with JAG1 staining shown in green. Analysis of the TMA reveals that **(B)** JAG1 is higher in the primary tumor of patients with positive LNs and **(C)** 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: Generation of Five Knockout JAG1 Cell Lines Using CRISPR/Cas9

A



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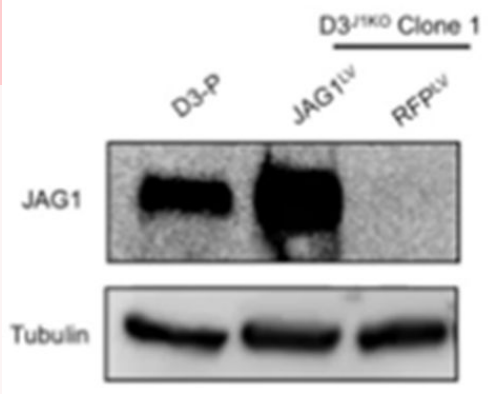

Figure 2. (A) Two separate guide RNAs (sgRNAs) targeting Exon2 of JAG1 were used to establish five distinct JAG1 knockout clonal lines from the parental D3H2LN line (D3-P), named $D3^{J1KO}$ clonal lines 1-5. The sgRNA names refer to position on Exon2. Homozygous frameshift mutations were confirmed via 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: JAG1 Promotes TNBC Lung Metastasis and Extravasation

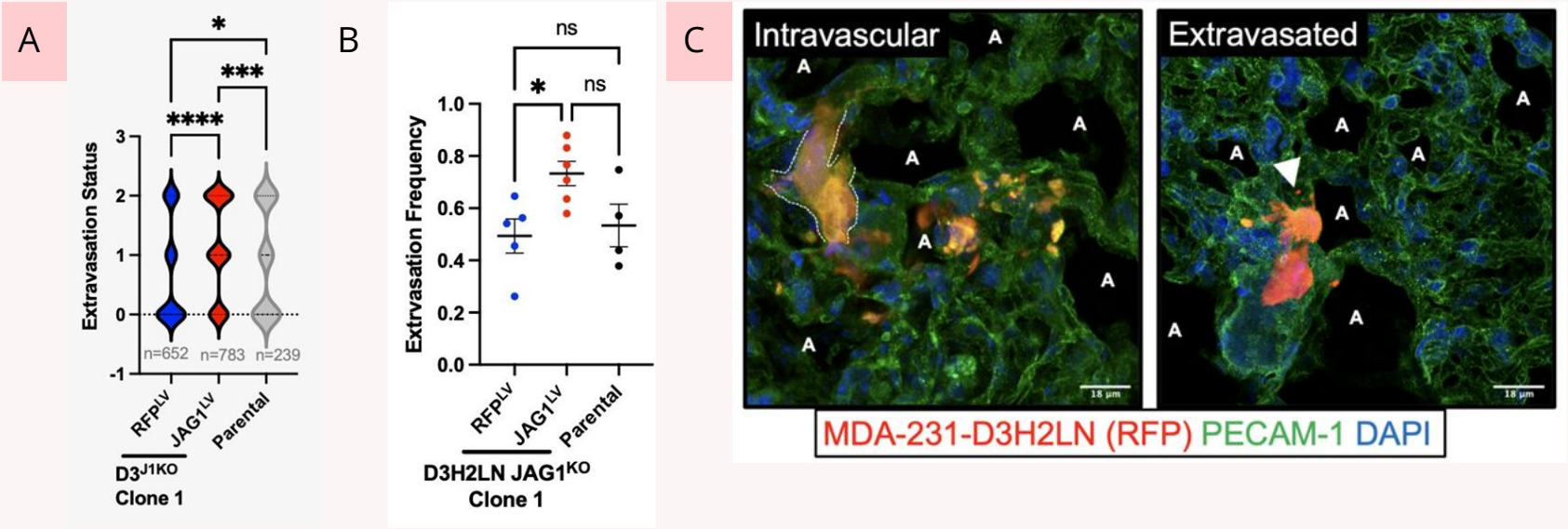

Figure 3. (A) Quantification imaging results shows JAG1 positive cells extravasate at a higher rate than knockout cells six hours after IV injection. n = each individual cell; 2 = extravasated; 1 = in-process; 0 = intravascular. **(B)** The extravasation frequency of cells is calculated by taking the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

04
CONCLUSION

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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MSTP

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