# Identification of Plasminogen Receptors of Secretome-stimulated Fibroblasts

Canadian Cancer Society / Société canadienne du cancer

Jordan Sampson

Supervised by: Dr. David Waisman and Gillian Okura

DALHOUSIE UNIVERSITY

## Introduction

- Metastasis drives the majority of breast cancer-related mortality [1]
- Primary tumours condition distant organs to form pre-metastatic niches (PMNs) [2]
- PMNs are permissive microenvironments that facilitate tumour colonization [3]
- Tumour-derived secretomes reprogram stromal cells into pro-metastatic phenotypes [3]
- Breast cancer cells reprogram fibroblasts to cancer-associated fibroblasts (CAFs) [4]
- Elevated plasminogen receptor expression in CAFs may represent a specialized subset with enhanced capacity to support PMN formation [5]
- Plasminogen is activated to protease plasmin via surface plasminogen receptors which co-localize plasminogen activators [6]
- Plasmin degrades the extracellular matrix (ECM), activates proteases, and releases pro-angiogenic growth factors, contributing to PMN formation [7]

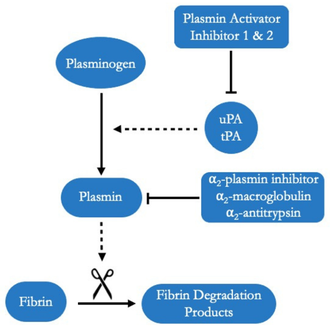



**Figure 1.** Overview of the plasminogen activation system.

### Knowledge Gap

Although tumour-derived secretomes reprogram fibroblasts and stromal plasminogen receptors are required for efficient plasmin generation during metastasis, these processes have not been directly linked.

### Hypothesis

Breast tumour secretomes from cell lines with distinct metastatic potential differentially prime the pre-metastatic niche by increasing plasminogen receptor (p11, p36, calreticulin) expression in lung fibroblasts.

## Methods

### Plasmin Generation Assays

MCF-7 and MDA-MB-231 breast cancer cells were cultured in serum-free media for 48 hours to generate tumour-derived secretomes. MRC-5 fibroblasts were seeded at 8,000 cells/well and treated with secretomes for 24 or 72 hours. Control wells received serum-free media. Assays were performed with three technical and three biological replicates per condition at each time point.

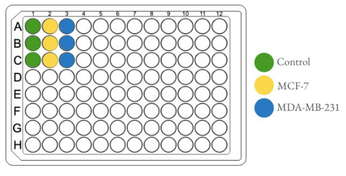



**Figure 2.** Experimental layout of secretome treatments used for plasmin generation assays.

### Data Analysis

Plasmin activity was calculated as the linear slope (ΔA405/min) and normalized to fibroblast number. Statistical significance was determined using a one-way ANOVA followed by Tukey post hoc tests where appropriate (RStudio).

### Western Blot Analysis

Membrane-enriched lysates from treated MRC-5 fibroblasts (30 µg protein) were resolved by SDS-PAGE and probed for plasminogen receptors (p11, p36, calreticulin) and plasminogen activator uPA. Band intensities were normalized to β-tubulin and quantified using ImageJ.

## Results

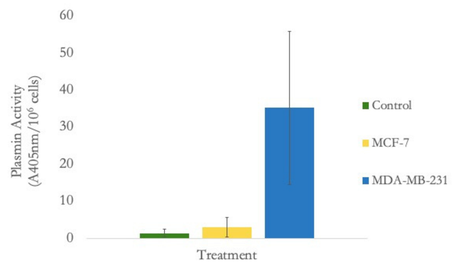



**Figure 3.** Plasmin activity in MRC-5 lung fibroblasts following 24-hour secretome treatment was significantly increased in the MDA-MB-231 condition compared to control and MCF-7 (one-way ANOVA p = 0.023; Tukey post hoc).

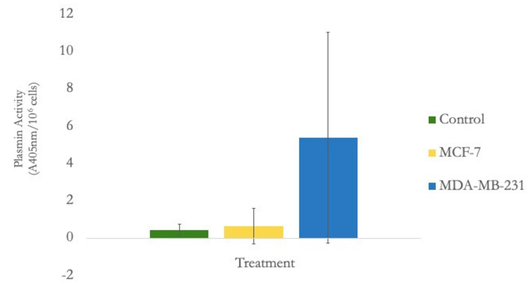



**Figure 4.** Plasmin activity in MRC-5 lung fibroblasts following 72-hour secretome treatment did not differ significantly between conditions (one-way ANOVA p = 0.20).

## Discussion

### 24-hour Response

- MDA-MB-231 secretomes significantly increased fibroblast plasmin activity
- MCF-7 treated fibroblasts and control fibroblasts did not differ in plasmin activity
- Plasmin generation appears cell line dependent, with the higher metastatic potential MDA-MB-231 line inducing greater stromal activation
- Suggests aggressive tumour-derived secretomes more effectively promote priming of fibroblasts

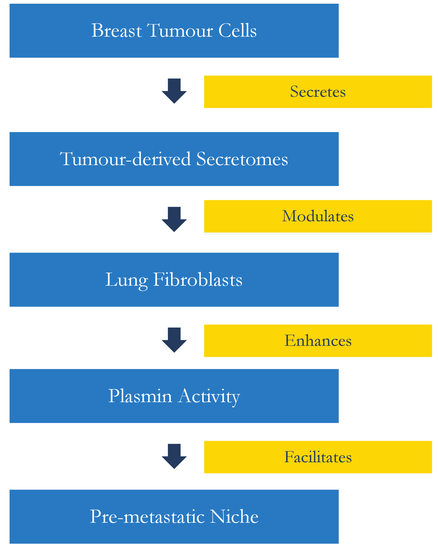
### 72-hour Response

- No significant differences observed
- Directional trend consistent with 24 h response, though not statistically significant
- Elevated variability across biological replicates
- Variability may reflect limitations of prolonged culture conditions or a transient activation response

## Conclusion

This study showed that secretomes from a highly metastatic breast cancer cell line significantly enhance fibroblast plasmin activity at 24 hours, supporting our hypothesis that tumour-derived secretomes functionally prime fibroblasts to increase stromal plasmin activation. Ongoing mechanistic analyses will determine whether these functional changes correspond to an upregulation in plasminogen receptors, plasminogen activators, or both.

## Proposed Model




## Future Direction

### Mechanistic Validation

- Determine whether increased plasmin activity corresponds with receptor and/or activator upregulation
- Assess plasminogen receptor (p11, p36, CRT) and plasminogen activator (uPA) expression

### Temporal Characterization

- Characterize the time course of secretome-induced plasmin activation
- Assess earlier and intermediate time points (e.g., 6 h, 12 h, 36 h)

### Model Expansion

- Validate findings in additional breast cancer cell line models
- Investigate whether stromal plasmin activation contributes to PMN formation in vivo

## References

[1] Canadian Cancer Society. 2017. Canadian Cancer Society. https://cancer.ca/en/.

[2] Kaplan RN et al. 2005. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. https://doi.org/10.1038/nature04186.

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[7] Bharadwaj AG et al. 2020. S100A10 Has a critical regulatory function in mammary tumor growth and metastasis: insights using MMTV-PyMT oncomice and clinical patient sample analysis. MDPI open access journals. https://doi.org/10.3390/cancers12123673.