Understanding the role of RHAMM in tumor load– mediated tumor invasiveness of Triple Negative Breast Cancer

Ashwin Sritharan[1], Britney Messam[2], and Eva Turley[2,3]

[1]Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1
[2]Department of Biochemistry, University of Western Ontario, London, Ontario, Canada, N6A 5C1
[3]London Regional Cancer Program, London, Ontario, Canada, N6A 4L6

Introduction

Breast cancer manifests heterogeneously in both clinical and molecular contexts, accounting for 25% of new cancer cases for women in Canada. While improvements in multi-modal therapy and population screening programs have increased survival dramatically[1], breast cancer remains as the second leading cause of cancer mortality in women in North America due to its high overall incidence.

In particular, Triple Negative Breast Cancer (TNBC) is particularly threatening due to rapid local invasive growth, metastasis, and chemotherapeutic resistance. While PARP and immune checkpoint inhibitors have proven useful for treating specific subsets of TNBC, effective therapies are still lacking for the majority of these patients[2].

The Turley and collaborator's laboratories (MJ Bissell, LBNL; JB McCarthy, UMN; and A Nelson, UMN) have shown that high expression of an HA receptor,

HMMR (aka *RHAMM*) is linked to poor outcome in TNBC cell subsets and is a critical factor in the experimental progression and metastasis of TNBC.

Previous studies suggest that RHAMM drives TNBC progression through an association with HA, the HA receptor CD44 and pro-migration/invasion signaling regulated by ERK1,23,4. TNBC cell lines lacking RHAMM expression via CRISPR-Cas9 gene editing are poorly invasive and metastatic in both orthotopic and lung colonization assays.

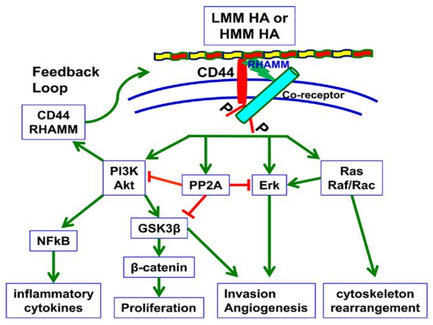


Figure 1. Activation of CD44, Erk, and Tumor Invasion and Proliferation via RHAMM-Dependent Pathway[7]

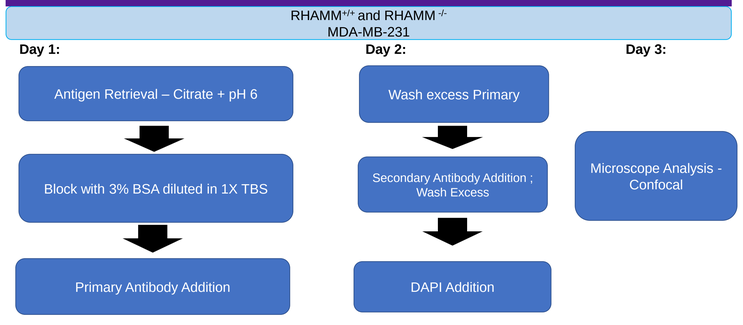
Hypothesis and Objectives

Hypothesis: RHAMM expression promotes invasive growth, proliferation of tumor cells and chemotherapeutic resistance in TNBC

Specific Objectives:

1. Compare the levels of proliferation between RHAMM+/+ and RHAMM -/- MDA-MB-231 spheroids as determined by ki67 and Caspase 3 signaling
2. Compare levels of RHAMM, CD44, Has2, and p-ERK activation between RHAMM+/+ and RHAMM -/- MDA-MB-231 spheroids

Methods



Results

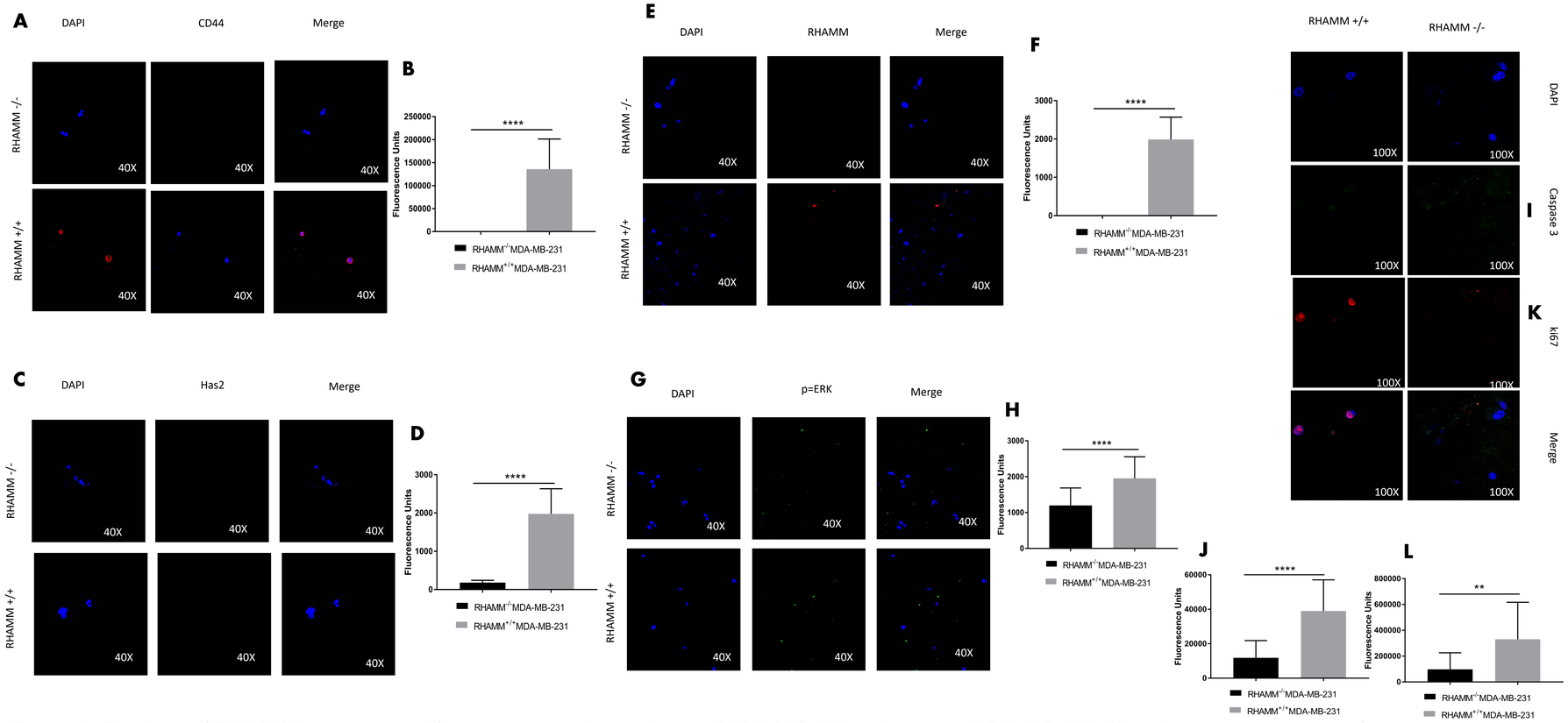


Figure 1. The loss of RHAMM reduces proliferation and cell signaling in MDA-MB-231 spheroids. MDA-MB-231 3D spheroids were grown for seven days and processed into paraffin-embedded sections. Representative images of RHAMM$^{+/+}$ and RHAMM$^{-/-}$ MDA-MB-231 spheroid sections stained with (**A**) CD44 (red), (**C**) HAS2 (red), (**E**) RHAMM (red), (**G**) p-ERK (green), (**I**) Cleaved Caspase 3 (green) and (**K**)) Ki67 (red) antibodies and counter-stained with DAPI (blue). Fluorescence quantification of (**B**) CD44, (**D**) HAS2, (**F**) RHAMM, (**H**) p-ERK, (**J**) Cleaved Caspase 3 and (**L**) Ki67 expression measured in ImageJ. Significant values are $p<0.01$ (**) and $p<0.0001$ (****) as determined by unpaired student's t-tests. Data represent mean ± SD.

Discussion and Future Directions

Discussion:

- As the presence of HA elevates CD44 expression, CD44 would be expected to be more highly expressed in RHAMM$^{+/+}$ MDA-MB-231
- Caspase 3 may be more highly expressed in RHAMM$^{+/+}$ MDA-MB-231 due to HA-mediated promotion of apoptosis
- RHAMM is required for translocation of p-ERK, so p-ERK expression is expected to increase in RHAMM$^{+/+}$ MDA-MB-231[5,6]
- Since Has2 has hyaluronan-mediated interactions with RHAMM, RHAMM expression could thus trigger Has2 expression

Future Direction:

- Quantification via qT-PCR/RT-PCR of markers presented could affirm findings presented
- Assessing expression of markers presented before and after exposure to anti-tumor therapeutics could provide novel targets for TNBC

References

[1]Berry et al 2005 *N Engl J Med* **367**,1998;
[2]Vagia et al 2020 *Cancers* **12**, 916;
[3]Liu et al., 2019 *Front Immunol* **10**:947;
[4]Veiseh et al 2014 *Proc Natl Acad Sci* **111**,1731;
[5]Zhang et al 1998 *J Biol Chem* **273**, 11342;
[6]Tolg et al 2006 *J Cell Bio* **175,** 1017;
[7]Jordan et al 2015 *Front Immunol* **6**, 182

Acknowledgments

All this work would not be possible without:
Dr. Eva Turley
Cancer Research Society
Breast Cancer Society of Canada
Western
Lawson Health Research Institute