Investigating the role of Lipocalin2 in circulating tumor cell dissemination

Sarah Huang[1], Ami Yamamoto[1,2], Brad Krajina, PhD[1], Kevin Cheung, MD[1]

[1]Translational Research Program, Public Health Division, Fred Hutchinson Cancer Research Center; [2]UW Molecular and Cellular Biology Graduate Program

contact: yhuang2@fredhutch.org ; ayamamo2@fredhutch.org

Background

Although advancements in the management of early stage breast cancer have improved patient survival overall, metastatic disease remains incurable.[1,2] Circulating tumor cells (CTCs) and clusters are indicators of cancer cell dissemination and are independently associated with poorer clinical outcome. Therefore, we are interested in studying the mechanism(s) regulating CTC dissemination.

CTC counts correlate with percent necrotic area in primary tumor

4T1-eGFP organoids were orthotopically transplanted into immunocompromised (SRG) rats. Primary tumors and blood were harvested at four different time points post transplantation.

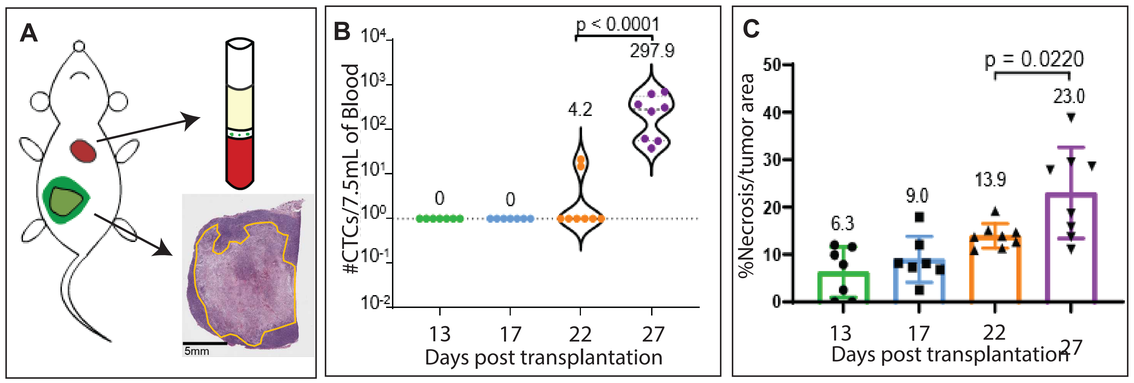

Fig 1. Circulating tumor cells (CTCs) and cluster dissemination correlate with percent necrosis in primary tumor: **A)** Experimental schematic;**B)**Total CTC counts from buffy coat per 7.5mL of blood;**C)** Percent necrosis area in primary tumor quantified from scanned H&E tumor sections.

Intravasated CTCs are commonly found in perinecrotic regions

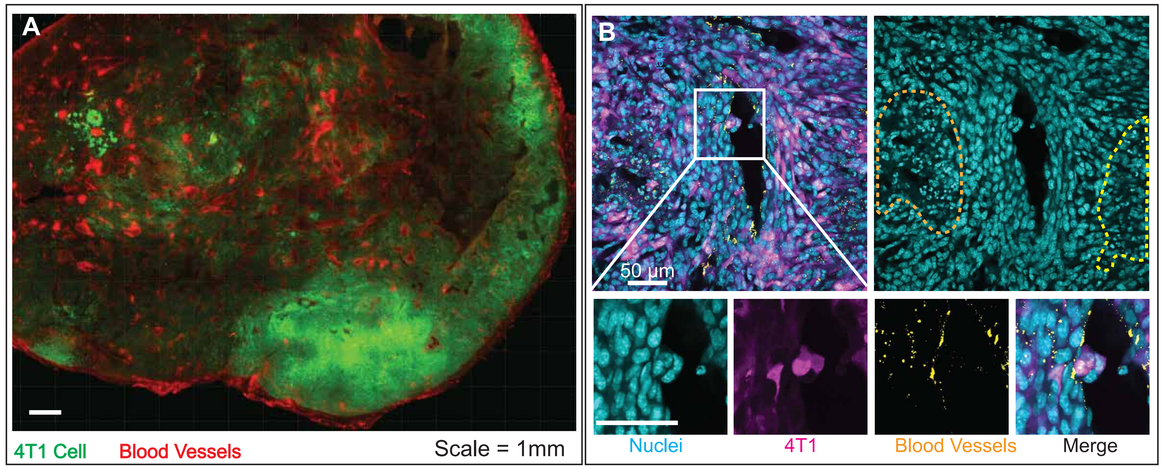

Fig 2. Intravasated CTCs are more commonly found in perinecrotic regions: **A)** 4T1 transplanted primary tumor with blood vessels labeled with intravenously administered fluorescent-conjugated lectin. Higher density of blood vessels observed in perinecrotic regions; **B)** Intravasation of CTCs and CTC clusters (magenta) is observed more frequently in blood vessels (yellow) located in perinecrotic areas. (Necrotic region is enclosed by dotted yellow line). Inset: magnified images of intravasating cancer cell cluster.

Lipocalin2 is highly expressed in necrotic region

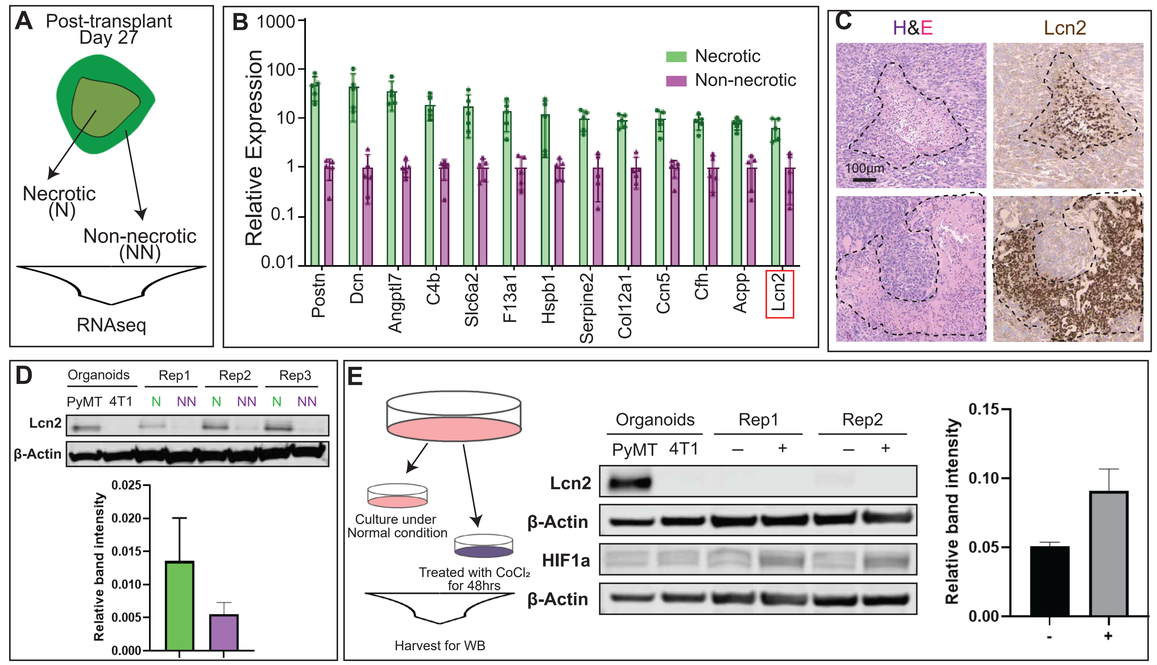

Fig 3. Lipocalin2 expression is concentrated in regions of necrosis. A) Experimental schematic; B) Transcriptional profile reveals that Lcn2 is highly expressed in necrotic regions; C) Lcn2 expression is observed in necrotic cores (enclosed by dotted black lines) by IHC; D) Immunoblots also quantitatively confirm Lcn2 location. Bottom: quantification of Lcn2, n=3; E) Culturing 4T1 cells in the presence of cobalt chloride for 48hrs induces HIF1a expression but not Lcn2, n=2.

Summary

Conclusions:

1. Percent tumor area of necrosis is positively— correlated with increased CTCs dissemination;
2. Higher blood vessel density is observed in perinecrotic regions than non-necrotic areas;
3. CTCs are more frequently observed intravasating into blood vessels in perinecrotic regions;
4. Transcriptional profiling with RNAseq reveals up-regulation of Lcn2 expression in necrotic regions;
5. Immunohistochemistry and immunoblots verified high Lcn2 protein expression located in regions of necrosis;
6. Treating 4T1 cells with cobalt chloride induced HIF1a expression but not Lcn2 expression, which might suggest that inducing HIF1a expression alone is not sufficient to induce Lcn2 expression.

Future directions:

1. Quantify the differences in vascular density and CTC intravasation events between necrotic and non-necrotic regions;
2. Evaluate the effects of loss of Lcn2 expression on 4T1 tumor outgrowth and metastasis in the rat transplant model.

Reference:

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