Manipulation of epithelial-to-mesenchymal transition reveals metastatic breast cancers support immune suppression via heme metabolism

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Introduction

➢ Triple negative breast cancer (TNBC) has often undergone oncogenic epithelial-to-mesenchymal transition (EMT).
➢ EMT in BC models is associated with increased immune suppression and altered metabolism.

Project Model

A. Role of HO-1 in metastatic BC immune suppression

B. HO-1 expression and effector capacity, dead cell engulfment, were observed by flow cytometry and IncuCyte live cell imaging (EisenBio).

C. HO-1 was depleted in 66Cl-4 BC mammary carcinoma cells using shRNA. 66Cl-4 shHO1 cells were then injected orthotopically into immunocompetent hosts and we assessed primary tumor growth and lung metastatic capacity.

Hypothesis

We postulate that tumor cell HO-1 activity and subsequent bilirubin secretion enhance TNBC metastasis by supporting a pro-tumor immune microenvironment.

Methods

➢ We assessed immune suppressive and effector genes in RAW264.7 mouse macrophages via qRT-PCR after direct treatment with bilirubin or treatment with conditioned medium (CM) of HO-1 inhibited mammary carcinoma cells.
➢ Macrophage PD-L1 expression and effector capacity, dead cell engulfment, were observed by flow cytometry and IncuCyte live cell imaging (EisenBio).
➢ HO-1 was depleted in 66Cl-4 BC mammary carcinoma cells using shRNA. 66Cl-4 shHO1 cells were then injected orthotopically into immunocompetent hosts and we assessed primary tumor growth and lung metastatic capacity.


Graphical Abstract: HO-1-mediated EMT induces HO-1 in BC cells. This project demonstrates HO-1 supports immune suppression by metastatic BC cells via its metabolite bilirubin.

Graphical Abstract: 2.5 µM bilirubin secreted by HO-1 expressing BC cells increased HO-1 and bilirubin expression in mouse peritoneal macrophages.

Fig 1. Tumor cell-HO-1 alters immune suppressive and effectorcytosis macrophage genes via secreted bilirubin.

A. Experimental design

B. RAW264.7 Macrophage Gene Expression

C. Macrophage Effector Activity

Fig 2. Bilirubin enhances PD-L1 expression in human-derived and primary mouse macrophages.

A. Experimental Design

B. PD-L1 Gene Expression

C. PD-L1 Protein Expression

Fig 3. BR decreases mouse and human macrophage engulfment of dead cells (effectorcytosis capacity).

A. Experimental Design

B. Macrophage Effector Activity

C. RAW264.7, 24h

Fig 4. Inhibition of tumor cell-HO-1 limits lung metastatic capacity.

A. HO-1 Knockdown

B. IHC Primary Tumors

C. Tumor Volume

D. Lung Metastatic Capacity

Fig 5. HO-1 correlates with markers of a suppressed tumor microenvironment.

A. IHC Primary 66Cl-4 Tumors

B. CIBERSORT TCGA Breast Cancer Specimens

C. microRNA

D. CD T Cells

Conclusions/Future Directions

➢ Restoration of mif-200c revealed heme metabolism via HO-1 as a possible immune modulatory pathway in breast cancer.
➢ Follow-up analysis demonstrated tumor cell-HO-1 inhibition altered expression of macrophage immune suppressive and effectorcytosis genes via bilirubin.

Future studies will continue to test the impact of HO-1 inhibition (shRNA or SmNIP) on immune suppressive T cells (exp. Treg) and macrophage function (exp. expression of effectorcytosis receptors) in experimental metastasis models to the lung and liver.

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Reference


Fig 1. Macrophages were cultured with and without 2.5 µM BR delivered in control or bilirubin-depleted conditioned medium (CM) collected from mammary carcinoma cells treated with 10 µM tin mesoporphyrin (SnMP).

Fig 2. A. RAW264.7 and bone marrow-derived (BMDC) and THP-1 human-derived macrophages were treated with a dose escalation of BR for 48 hours (0.25 µM), selected doses were chosen for further study (B). Gene (B) and protein (D) expression of PD-L1 was assessed by qRT-PCR and flow cytometry. 100 ng/mL IFNγ + LPS was a positive control.

Fig 3. A-C. RAW264.7 and BMDC mouse and THP-1 human-derived macrophages were treated with 2.5 ± 10 µM BR for 0-24 hours (A). Effectorcytosis was measured using an IncuCyte effectorcytosis assay (B) where dead 66Cl-4 cells were dead with a marker that fluoresces bright red in the high pH environment of macrophage lysosomes (C).

Fig 4. A. HO-1 was genetically depleted from 66Cl-4 mammary carcinoma cells (shHO1) that were then injected into the mammary bed of syngeneic BALB/c mice in a primary study; B-C. 8 weeks later, primary tumors were treated for HO-1 expression via IHC (B), and tumor volume (C) and metastatic capacity (D) were assessed.

Fig 5. A. IHC conducted on 66Cl-4 HO-Flox/+ mammary tumors for FoxP3 (regulatory cell marker) and Arg1 (immune suppressive macrophage marker). CIBERSORT analysis conducted on breast cancer specimens from the Nature 2012 dataset stratified based on expression of heme metabolism genes (NRAS3Cl and ELRP3).