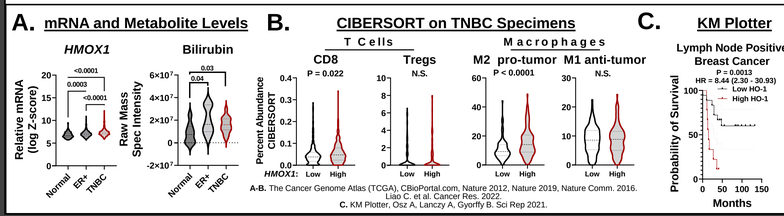
Breast Tumor Cell Heme Metabolism Alters Macrophage Immune Suppression and Function to Support Lung Metastasis

Michelle M. Williams[1]; Sabrina A. Hafeez[1]; Jessica L. Christenson[1]; Nicole S. Spoelstra[1]; Kathleen I. O'Neill[1]; Li-Wei Kou[1]; Lyndsey S. Crump[1]; Jill E. Slansky[2]; Jennifer K. Richer[1]

University of Colorado, School of Medicine, Department of Pathology[1] or Department of Immunology and Microbiology[2]

Introduction/Rationale

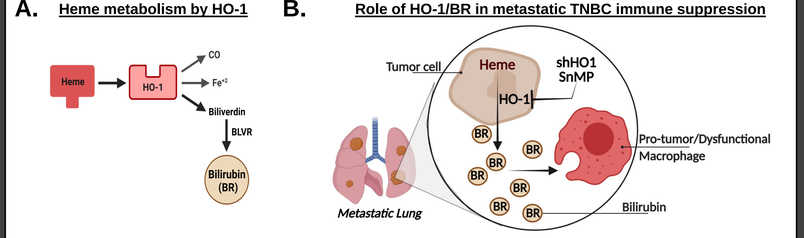
- Heme oxygenase-1 (*HMOX1*/HO-1) expression and activity are elevated in triple-negative breast cancer (TNBC) specimens (**A**).
- HO-1 metabolizes heme into bilirubin, a metabolite known to impact macrophage function in autoimmune diseases. (Liu Y. et al. J Immunol. 2008)
- Even though HO-1 expression predicts increased abundance of suppressive immune cells (**B**) and decreased overall survival (**C**); however, **the impact of the bilirubin on the TNBC tumor microenvironment had not been studied.**



Hypothesis

We **hypothesized** that tumor cell-HO-1 activity and subsequent bilirubin secretion enhance triple-negative breast cancer (TNBC) metastasis by supporting immune suppressive, pro-tumor macrophage function.

Project Model



Project Model. A. HO-1 metabolizes heme into three immune modulatory products, iron (Fe^{+2}), carbon monoxide (CO) and biliverdin that is converted to bilirubin (BR) by biliverdin reductase (BLVR). **B.** This project tests the impact of HO-1 on TNBC progression via BR-mediated immune suppression. We assessed the effects of BR on macrophage function by limiting tumor cell-HO-1 activity via the FDA approved HO-competitive inhibitor tin mesoporphyrin (SnMP) or shRNA (shHO1).

Methods

- I assessed immune suppressive and efferocytosis genes in RAW264.7 mouse macrophages via qRT-PCR after direct treatment with bilirubin or treatment with conditioned medium (CM) from HO-1 inhibited mammary carcinoma cells.
- Macrophage PD-L1 expression and efferocytic capacity, dead tumor cell engulfment, were observed by flow cytometry and IncuCyte live cell imaging (Essen Bio).
- HO-1 was depleted in 66Cl-4 mammary carcinoma cells using shRNA. 66Cl-4 shHO1 cells were injected orthotopically into immune-competent hosts and I assessed primary tumor growth, lung metastatic capacity, and macrophages status via flow cytometry.

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

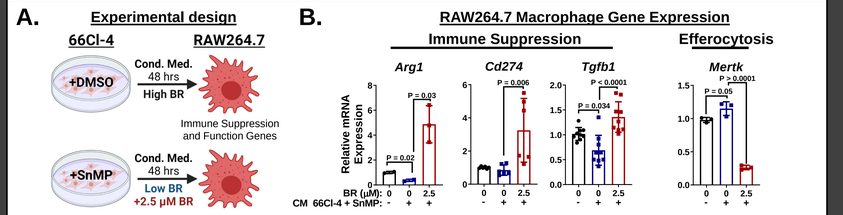


Figure 1 A. Macrophages were cultured ± 2.5 μM BR delivered in control or bilirubin-depleted conditioned medium (CM) collected from mammary carcinoma cells treated with 10 μM tin mesoporphyrin (SnMP). **B.** Immune suppressive (*Arg1, Cd274*/PD-L1, *Tgfb1*) and functional efferocytosis (*Mertk*) gene levels were determined via qRT-PCR (N = 3-9).

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

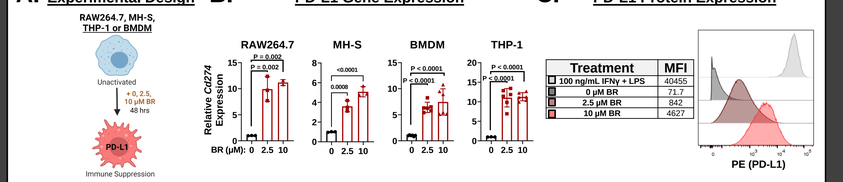


Figure 2. A. RAW264.7, MH-S and bone marrow-derived (BMDM) mouse, and THP-1 human-derived macrophages were treated with a dose escalation of BR for 48 hours (0-20 μM, select doses are shown). **B-C.** Gene (**B**) and protein (**C**) expression of PD-L1 was assessed by qRT-PCR and flow cytometry. 100 ng/mL IFNγ + LPS was a positive control (N = 3-6).

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

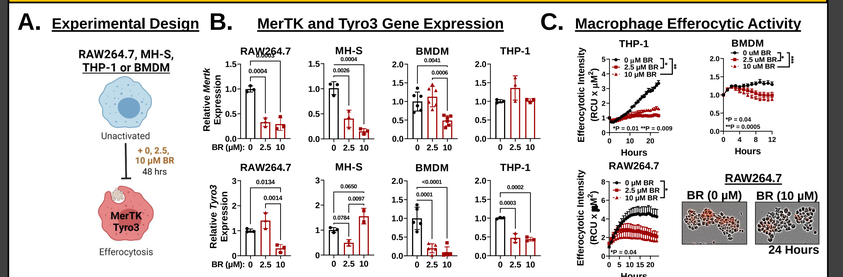


Figure 3. A. RAW264.7, MH-S and BMDM mouse, and THP-1 human-derived macrophages were treated with 0-10 μM BR for 0-24 hours (select doses are shown). **B-C.** Efferocytosis gene expression (**B**) and capacity (**C**) were measured. An IncuCyte assay was used. Dead 66Cl-4 cells were dyed with a marker that fluoresces bright red in the high pH of lysosomes (**C**, bottom right, N = 3-12).

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

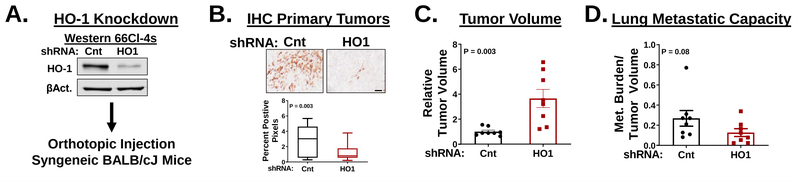


Figure 4. A. HO-1 was genetically depleted from 66Cl-4 mammary carcinoma cells (shRNA) that were then injected into the mammary fatpads of syngeneic BALB/cJ mice in a preliminary study. **B-C.** 8 weeks later, primary tumors were analyzed for HO-1 expression via IHC (**B**), and tumor volume (**C**) and metastatic capacity (**D**) were assessed (N = 8).

Fig 5. HO-1 supports macrophage expression of suppressive markers and immunotherapy resistance.

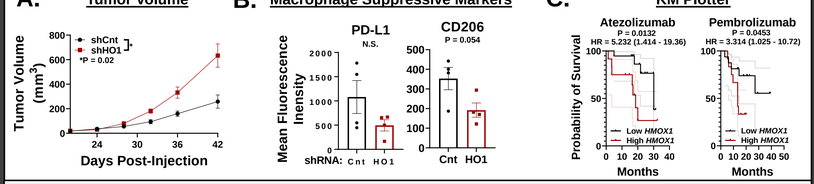


Figure 5. A-B. Repeat of mouse experiment from Fig. 4. Shown are tumor volume (**A**) and flow cytometry conducted on tumor infiltrating macrophages in primary tumors (N = 4-8, **B**). **C.** KM Plotter observing expression of *HMOX1* in cancer patients treated with Atezolizumab or Pembrolizumab (N = 28-32).

Conclusions/Future Directions

- HO-1 expression and activity is increased in TNBC specimens, and it predicts poor overall survival, a suppressed microenvironment, and decreased response to immunotherapy.
- Tumor produced bilirubin increased macrophage expression of PD-L1 but decreased efferocytic capacity by MerTK and Tryo3.
- In metastatic models, inhibition of tumor cell-HO-1 decreased macrophage expression of suppressive markers at the primary site and decreased lung metastatic capacity.
- Future studies will continue to test the impact of HO-1 inhibition (shRNA or SnMP) on T cell cytotoxicity and macrophage immune suppression and function in the metastatic lung and liver.
- ***Summary**: HO-1 targeting with FDA approved SnMP may limit macrophage immune suppression via bilirubin depletion, resulting in decreased TNBC metastasis and enhanced sensitivity to immunotherapy.*

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