Determining the Role of Inflammatory Signaling on B7H3 Expression for Triple-Negative Breast Cancer

Hannah M. Oh[1], Hannah M. Malian[1], Alyssa N. Ho[1], Michael F. Coleman[1], Stephen D. Hursting[1,2,3]

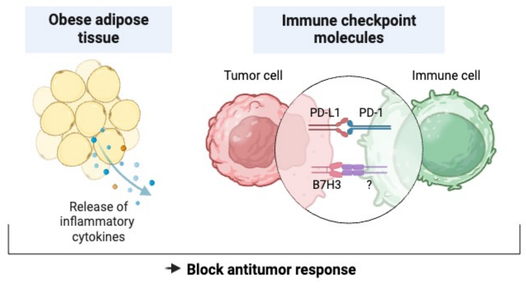
1. Department of Nutrition, University of North Carolina (UNC), Chapel Hill, NC; 2. Nutrition Research Institute, UNC, Kannapolis, NC; 3. Lineberger Cancer Center, UNC, Chapel Hill, NC

Significance and Rationale

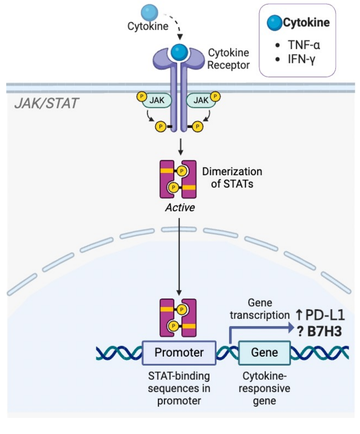
- Obesity is an established risk factor for breast cancer
- Understanding the regulatory mechanisms for B7H3 expression will open the door for B7H3-targeting immunotherapy options

Introduction

- Within the tumor microenvironment, obesity-associated inflammatory signaling results in immunosuppression of T cells, promoting tumor growth
- B7H3, a known immunosuppressive protein, is highly expressed in triple negative breast cancer (TNBC) and is correlated with poor prognosis



Study Design

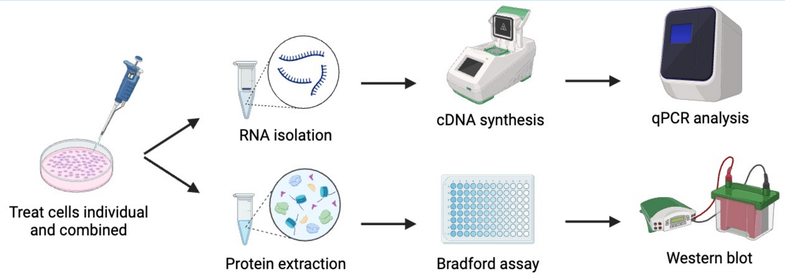


- Pro-inflammatory cytokines bind to receptors on cancer cells
- Cytokines signal downstream targets, upregulating immunosuppressive proteins
- IFN-γ and TNF-α upregulate PD-L1, an immunosuppressive protein in the B7 family
- Strong need to investigate the impact of pro-inflammatory cytokines on the regulation of B7H3 (also in the B7 family)

Hypothesis

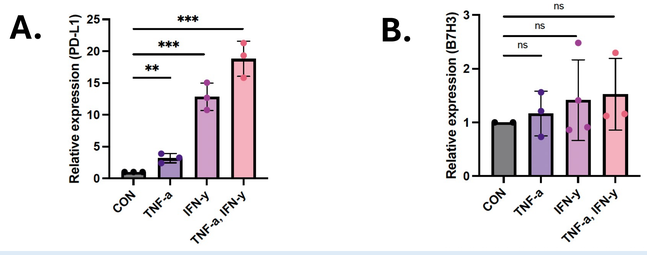
Inflammatory cytokines, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), will promote B7H3 expression in TNBC cells via JAK/STAT and/or NFKB signaling.

Methods



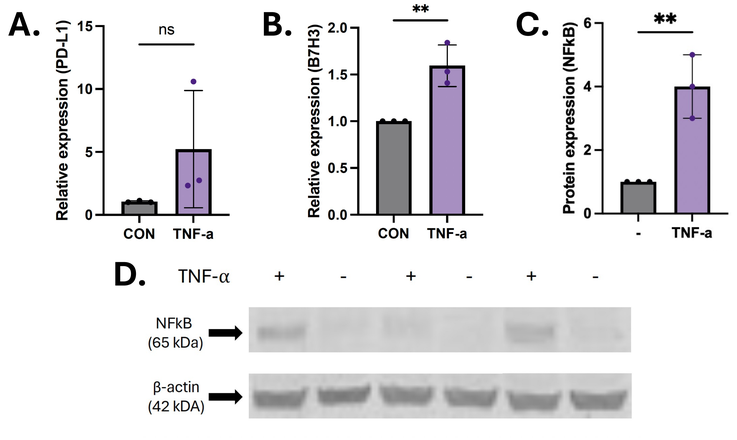
Methods. E0771 cells (murine TNBC cell line) were seeded in DMEM media with 10% FBS, 1% Pen-Strep, 1% L-glutamine. After incubating overnight at 37°C, cells were washed 1x with PBS and treated alone or in combinations with IFN-γ (20 ng/mL) and TNF-α (20 ng/mL) for 4 and 24 hours in serum-free DMEM. Western blots and qPCR were conducted to examine PDL1 and B7H3 protein and RNA levels, respectively. STAT1 and NF-κB (P65) phosphorylation served as a positive control for signaling induction.

Preliminary Results



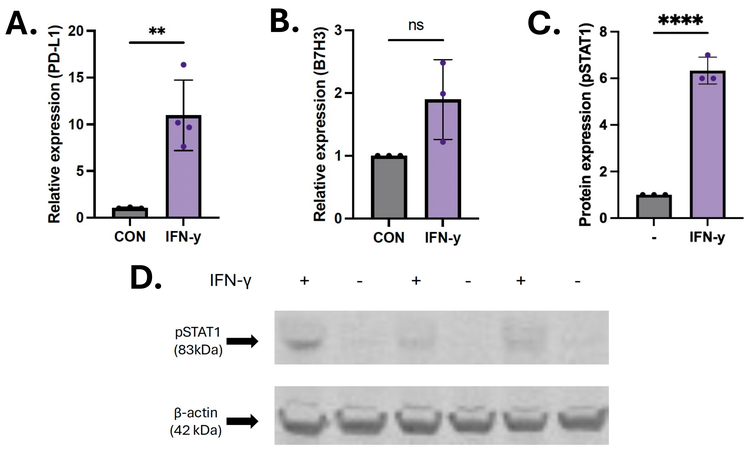
Cytokines upregulate PD-L1, but not B7H3, after 4 hours of treatment. qPCR analysis of (A) PD-L1 expression and (B) B7H3 expression after 4 hrs. of individual and combined cytokine treatment.

Results



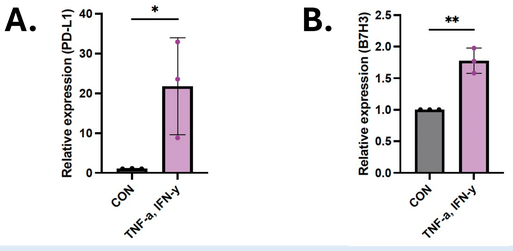
TNF-α increases B7H3 expression by 60% through the activation of NF-κB signaling *in vitro*. qPCR analysis shows upregulation of (A) PD-L1 and (B) B7H3 after 24 hrs. of TNF-α treatment. Western blotting reveals activation of NF-κB signaling pathway after 24 hrs. of TNF-α treatment (C & D) .

Results



IFN-γ increases B7H3 expression by 90% through the activation of STAT1 signaling *in vitro*. qPCR analysis shows upregulation of (A) PD-L1 and (B) B7H3 after 24 hrs. of IFN-γ treatment. Western blotting reveals activation of STAT1 signaling pathway after 24 hrs. of IFN-γ treatment (C & D) .

Future Directions



Combined treatment with IFN-γ and TNF-α increases B7H3 expression by 80% compared to the untreated control. qPCR analysis shows upregulation of (A) PD-L1 and (B) B7H3 after 24 hrs. of combined cytokine treatment.

Conclusions

- At 24-hour time points, individual treatment with IFN-γ or TNF-α significantly upregulates B7H3 expression in E0771 relative to untreated control
- B7H3 induction by inflammatory cytokine signaling may represent a novel axis through which obesity-driven inflammation may promote tumor progression
- Future directions: (1) test alternative time points examine dynamics of cytokine signaling (2) analyze the *in vitro* effects of combination cytokine treatment using western blots

Funding and Acknowledgements

This research was funded by the Breast Cancer Research Foundation (BCRF-23-073) and the UNC Triple Negative Breast Cancer Center. Illustrations made with Biorender.