Immune Checkpoint Inhibitors and 15dPMJ2's Effect on CHOP10 Expression in Syngeneic Murine Melanoma

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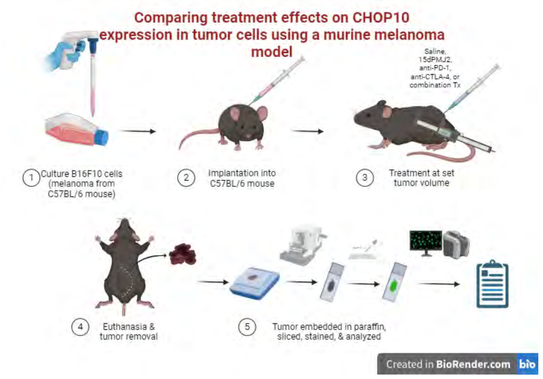
INTRODUCTION

- Immune checkpoint inhibitors (ICIs) targeting PD-1 and CTLA-4 are FDA approved to treat late-stage melanoma, but offer limited improvement in overall survival.
- Optimized and/or new therapies are needed to improve treatment response and long-term survival in melanoma patients.
- 15dPMJ2, a endocannabinoid prostamide metabolite, directly induces melanoma cell death by activating the ER stress pathway.
- 15dPMJ2 also triggers an adaptive immune response by promoting damage-associated molecular pattern (DAMPs) expression on tumor cell surfaces.
- If ICIs do not reduce its cytotoxic effects, 15dPMJ2 may improve melanoma tumor sensitivity to ICI therapy.

HYPOTHESIS

15dPMJ2 will promote CHOP10 expression and colocalization to the nucleus. ICIs alone will not promote CHOP10 expression but they will not hinder 15dPMJ2's ability to upregulate the proapoptotic transcription factor.

MATERIALS & METHODS



- Murine melanoma model (syngeneic, immunocompetent)
- 9 Treatment groups: Saline, IgG, 15dPMJ2, anti-PD1, anti-CTLA4, 15dPMJ2 + anti-PD1, 15dPMJ2 + anti-CTLA4, anti-PD1 + anti-CTLA4, & 15dPMJ2 + anti-PD1 + anti-CTLA4
- 18 tumors sectioned: 2 mice from each treatment group; one male and one female
- Immunofluorescence microscopy performed on 2-3 tumor sections per tumor; 4 fields imaged at 20x magnification, totaling 8-12 unique fields imaged per tumor

PRELIMINARY RESULTS

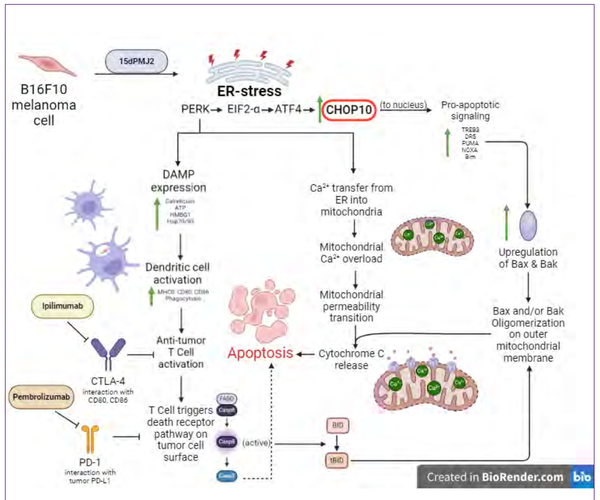


Figure 1: Schematic of the relationships between mechanisms of action for 15dPMJ2, ipilimumab (anti-CTLA4), and pembrolizumab (anti-PD1).

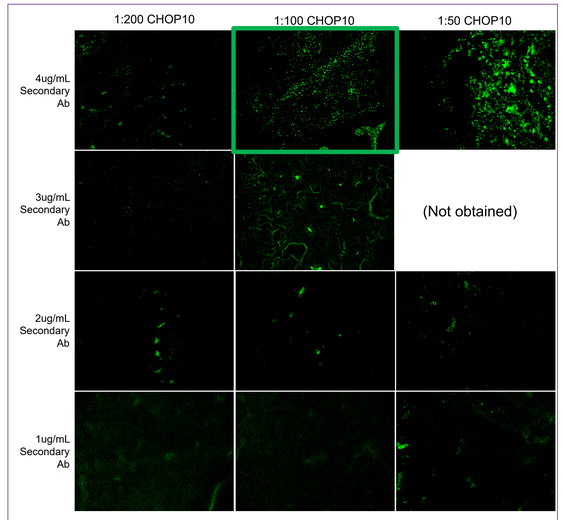


Figure 2: A 1:100 dilution of CHOP10 primary antibody and a 4ug/mL concentration of secondary antibody is optimal for detecting the presence of CHOP10 in melanoma cells.
The staining procedure described in Figure 1 was performed on 4 slides with 4 tissue sections each, from a tumor that had been treated with 15dPMJ2. .

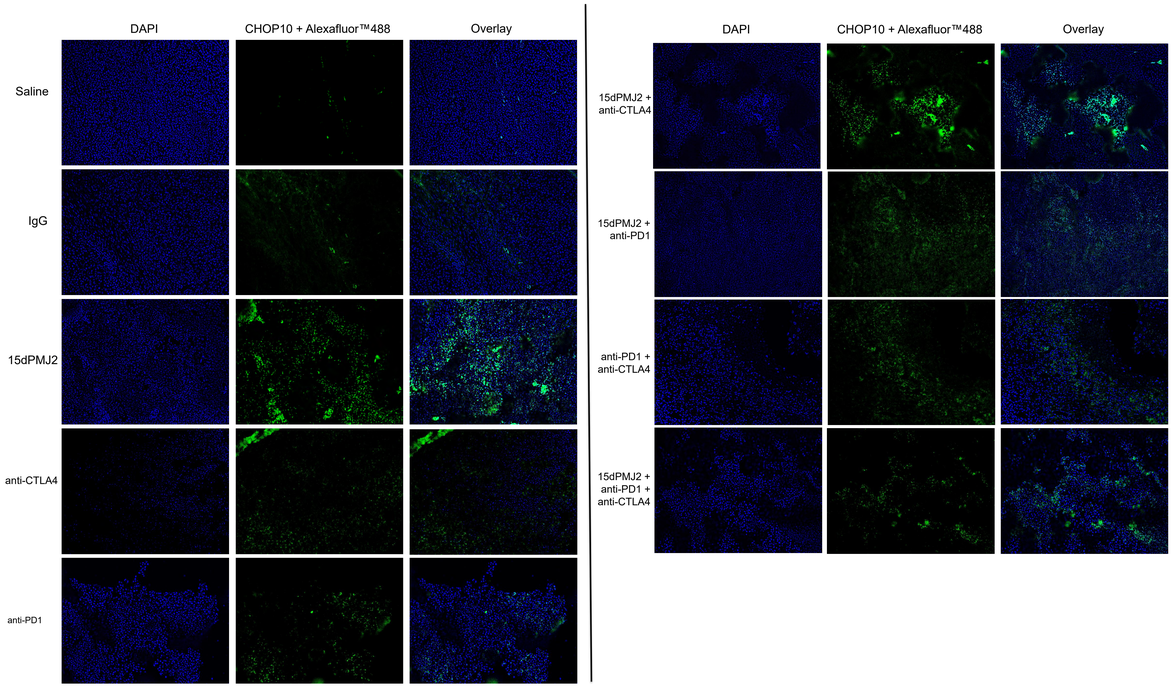


Figure 3: CHOP10 expression appears higher relative to vehicle groups when 15dPMJ2 is included in treatment regimen. Anti-PD1 + anti-CTLA4 combined treatment group appears to slightly increase CHOP10 expression, but as monotherapies neither appear to significantly affect CHOP10 levels.
Tumors removed from euthanized mice were placed in formalin for at least 48 hours, then embedded in paraffin blocks. Tumors were sectioned into 5um-thin slices and placed on microscope slides. Slides were then de-waxed and rehydrated, and 95-100ºC EDTA was used for antigen retrieval. Cells permeabilization was performed with PBS containing 0.25% Triton X-100. Tissues were stained with 1:100 dilution of anti-CHOP10 primary goat antibody overnight at 4ºC, then incubated in 4ug/mL anti-goat Alexafluor 488 secondary rabbit antibody for 2 hours at room temperature. Cell nuclei were stained with DAPI. Immunofluorescence microscopy was performed; four fields of 2-3 tissue slices were imaged per primary tumor, assessing CHOP10 fluorescence in the green channel and DAPI fluorescence in the blue channel. One additional tumor section on each slide was incubated without CHOP10 and served as a negative control to optimize exposure level for minimal background fluorescence.

MATERIALS & METHODS CONTINUED

- CHOP10 detected with goat primary antibody tagged with Alexa Fluor™ 488-labeled anti-goat secondary antibody
- DAPI stains AT-rich regions of DNA; allows for colocalization analysis of CHOP10 and nuclei

DISCUSSION

The initial qualitative impression from acquired immunofluorescence microscopy images is that tumors that received 15dPMJ2 as part of treatment have greater numbers of CHOP10 positive cells than those of tumors not treated with 15dPMJ2. While anti-CTLA4 and anti-PD1 do not appear on their own to have increased CHOP10 positive cells compared to tumors that received vehicle (saline or IgG) treatments, they do appear to induce CHOP10 expression when combined with one another. However, quantitative assessment will be necessary before conclusions can be made.

FUTURE DIRECTIONS

CHOP10/DAPI colocalization data will be obtained from collected images to determine if 15dPMJ2 increases the quantity of CHOP10 positive cells in melanoma tumors compared to treatments without 15dPMJ2.

TUNEL staining will be utilized to detect levels of DNA fragmentation in the tumor tissue from each treatment group. This will elucidate whether 15dPMJ2 may further increase tumor cell apoptosis when combined with ICIs.

Flow cytometry will also be performed to compare types and quantities of tumor-infiltrating lymphocytes (TILs) found in the tumors from each treatment group. Quantifying TIL types can indicate whether 15dPMJ2 may increase tumor sensitivity to ICI treatment

ACKNOWLEDGEMENTS

This work was supported by the Brody School of Medicine Summer Scholars Research Program and a Translational Research Grant from the North Carolina Biotechnology Center