15-Deoxy-Δ^{12,14}-Prostamide J$_2$ Induces PERK-IP3-Receptor Calcium Signaling and Mediates Mitochondrial Permeability Transition Pore Opening in Melanoma

Daniel A. Ladin[1], Margaret M. Nelson[2], Catherine Colonna[1], Jacques Robidoux[3], Kelsey Fisher-Wellman[2] and Rukiyah Van Dross[3]

[1]Department of Medicine, [2]East Carolina Diabetes and Obesity Institute, [3]Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University

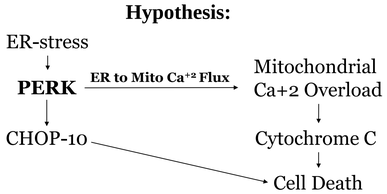
ABSTRACT

Melanoma is the deadliest form of skin cancer in the United States. Recent FDA-approved therapies for melanoma have increased patient survival. However, new or optimized therapeutic approaches are needed to improve treatment outcomes. 15-deoxy-Δ^{12,14}-prostamide J2 (15d-PMJ$_2$) is an investigational small molecule that induces ER stress-mediated apoptosis selectively in tumor cells.[1,2] Additionally, 15d-PMJ$_2$ significantly reduces melanoma growth *in vivo*.[1] The goal of this study was to investigate mechanisms underlying the antitumor activity of 15d-PMJ$_2$. We found that the ER stress sensor, PERK, was required for 15d-PMJ$_2$-induced apoptosis. PERK activation triggered the release of ER-resident Ca^{2+} through an IP$_3$R sensitive pathway. Increased calcium mobilization led to the accumulation of Ca^{2+} in the mitochondria followed by the induction of the mitochondrial permeability transition pore (mPTP) and the deterioration of mitochondrial respiration. Finally, we demonstrated that the electrophilic double bond located within the cyclopentenone ring of 15d-PMJ$_2$ was required for its activity. Taken together, the present study identifies PERK/IP3R/mPTP signaling as a mechanism of 15d-PMJ$_2$ anti-melanoma activity.

INTRODUCTION

- Melanoma is the deadliest form of skin cancer with roughly 7,000 individuals dying from the disease annually.
- 15-deoxy-Prostamide J$_2$ (15d-PMJ$_2$) is a novel anti-cancer agent that eliminates melanoma by inducing ER-stress mediated programmed cell death
- ER-stress occurs when unfolded proteins exceed the cellular folding capacity
- Activation of ER-stress is known to cause Ca^{+2} mobilization and subsequent induction of programmed cell death (apoptosis).
- Mitochondrial Ca^{+2} overload mediated through IP$_3$ receptor sensitive pathways causes cytochrome c release and results in cell death.

Hypothesis:



RESULTS

PERK is required for 15d-PMJ$_2$–induced ER stress apoptosis

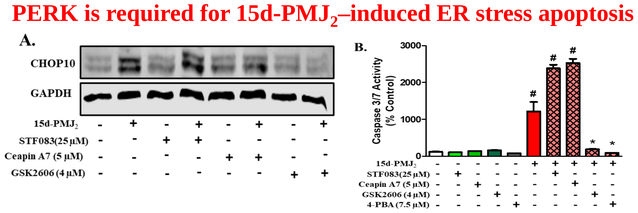


Figure 1. B16F10 cells were pretreated with ER-stress inhibitors for 30 minutes followed by treatment with 15d-PMJ$_2$ for 8 hours. The expression of CHOP10 was measured by conducting Western blot analysis after 8 hours of cell treatment with 15d-PMJ$_2$ or vehicle. (C, D) Caspase 3/7 activity was detected by utilizing Caspase Glo-3/7 reagents (Promega) Data represent mean ± SEM of three independent experiments and are expressed as percent of untreated group. # $P < 0.05$, as compared to vehicle, * $P < 0.05$, as compared to 15d-PMJ$_2$.

RESULTS

Activation of Ca^{2+} channels by 15d-PMJ$_2$ is mediated by ER-stress and required for cell death

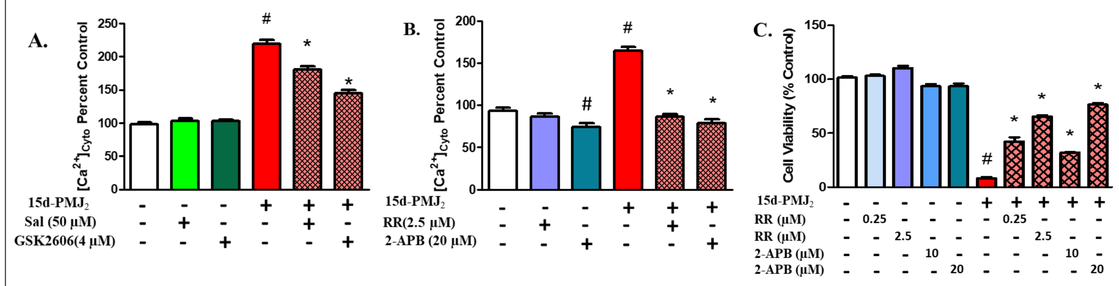


Figure 2. (A) B16F10 cells were pretreated with the ER stress inhibitors, salubrinal and GSK2606414, for 30 minutes followed by treatment with 5 μM 15d-PMJ$_2$ or vehicle. Cytoplasmic Ca^{2+} was measured by performing assays with Fluor-4 NW. (B) B16F10 cells were pretreated for 1 hour with the calcium channel blocker ruthenium red (RR) and the IP3 receptor inhibitor, 2-APB. The cells were then treated with 5 μM 15d-PMJ$_2$ or vehicle for 1 hour and cytoplasmic Ca^{2+} was measured. (D) Cells were pretreated with RR (0.25 or 2.5 μM) or 2-APB (10 or 20 μM) for 1 hour followed by cell treatment with 5 μM 15d-PMJ$_2$ or vehicle for 24 hours. Cell viability was determined by conducting MTS experiments. The data are presented as the mean ± SEM of three independent experiments.* $P < 0.05$, when comparing samples to 15d-PMJ$_2$-treated cells, # $P < 0.05$, when comparing samples to vehicle-treated cells

15d-PMJ$_2$ mediated ER-stress increases mitochondrial calcium flux and membrane permeability

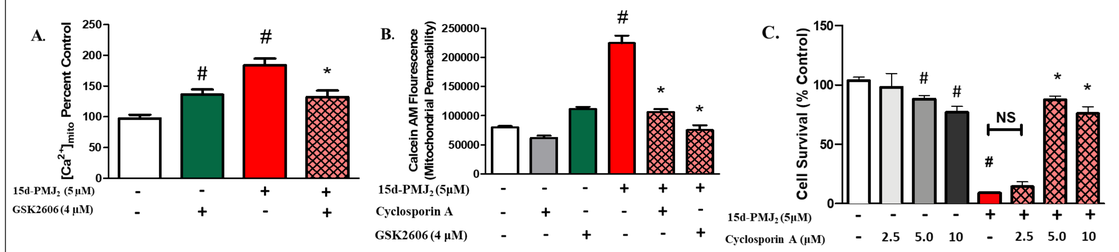


Figure 3. (A) B16F10 cells were pretreated with the ER-stress inhibitors (GSK, PBA) for 30 minutes, treated with 15dPMJ$_2$ or vehicle for 6 hours, and mitochondrial Ca^{2+} levels were determined by detecting Rhod-2 fluorescence by flow cytometric analysis. (B) B16F10 cells were pretreated with the mPTP inhibitor, cyclosporin (1 hour) or the ER stress inhibitors, GSK or 4-PBA and then the cells were treated with 15d-PMJ$_2$ or vehicle for 6 hours. Cell fluorescence was then quenched with $CoCl_2$ and assayed with Calcein-AM by flow cytometry. Data represent mean ± SEM of three independent experiments and are expressed as (A-C) percent of untreated group or (D) pmol/s/million cells. # $P < 0.05$, as compared to vehicle, * $P < 0.05$, as compared to 15d-PMJ$_2$.

15d-PMJ$_2$ decreases mitochondria respiration in a dose- and ER stress-dependent manner.

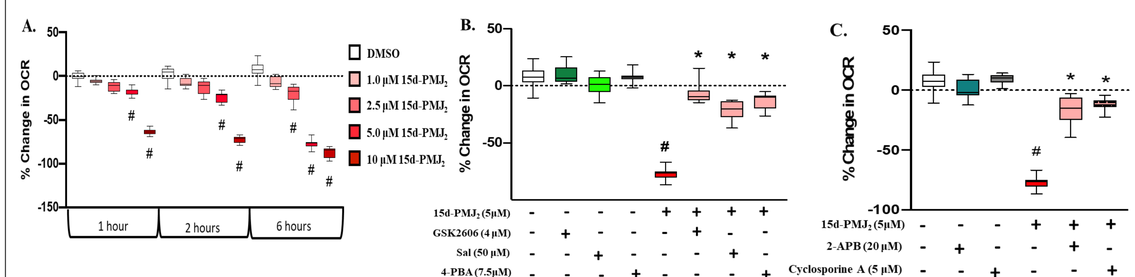
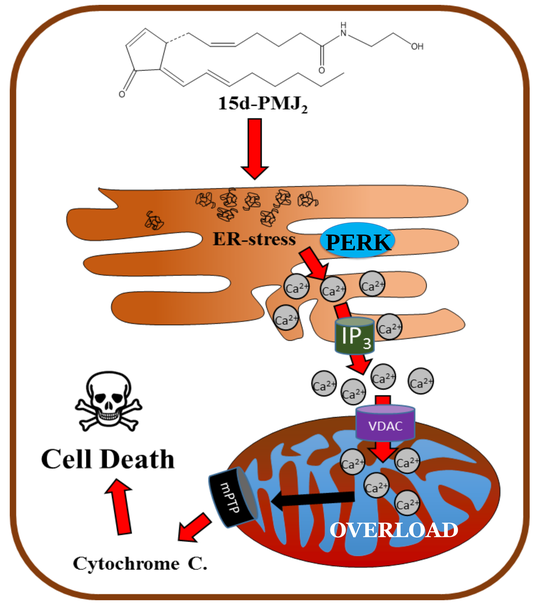


Figure 4. B16F10 cells were pretreated with different inhibitors for 30 minutes followed by incubation with 15d-PMJ$_2$. Mitochondrial oxygen consumption rate was measured using the Seahorse XFe96 Flux analyzer every 10 minutes for 6 hours. Data represent mean ± SEM of three independent experiments. # $P < 0.05$, as compared to vehicle, * $P < 0.05$, as compared to 15d-PMJ$_2$.

CONCLUSION

- PERK activity is necessary for the apoptoticaction of 15d-PMJ$_2$(figure 1)
- 15d-PMJ$_2$ increases cytoplasmic Ca^{+2} through ER-stress which is required for its cytotoxicity. (Figure 2)
- ER-stress induced by 15d-PMJ$_2$ increases mitochondrial Ca^{+2} levels and mPTP opening. (Figure 3)
- Activation of PERK-mediated ER-stress and subsequent Calcium flux results in significantly reduces mitochondrial respiration

Hypothesis of Mechanism



ACKNOWLEDGEMENTS

I would like to thank Ariel Myers, Kathleen Thayne, Maddie Craney and Emma Goldberg for their assistance in conducting this research. This research was supported by funds from: Brody Brothers foundation and UNC-Lineberger Cancer center pilot grant.

REFERENCES

1. Ladin, D. A. (2017) *Molecular Cancer Therapeutics*. **5**: (838-849)
2. Soliman, E. et. al. (2015) *Molecular Carinogensis*. In Press.