Polymer-Lipid Manganese Dioxide Nanoparticles Amplify Radiation Therapy-Induced Anti-tumor Immune Response in a Syngeneic Breast Tumor Model

Ho Yin Lip[1], Abdulmottaleb Zetrini[1], Azhar Z. Abbasi[1], Ping Cai[1], Andrew M. Rauth[2], and Xiao Yu Wu[1].
[1]Advanced Pharmaceutics and Drug Delivery Laboratory, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.
[2]Departments of Medical Biophysics and Radiation Oncology, University of Toronto, Toronto, ON, Canada.

Introduction

Immunogenic cell death (ICD) is a type of cell death that releases immunogenic damage-associated components (Fig. 1)[1].

The hypoxic tumor microenvironment (TME) alters cellular metabolism resulting acidification and oxidative stress and inhibits the establishment of an active immune response[2-4]. Macrophages become skewed towards immunosuppressive tumor-associated macrophages that promote tumor growth and inhibit T cell activity.

Our laboratory pioneered the bio-reactive polymer-lipid manganese dioxide nanoparticles (PLMDs) for TME modulation (Fig. 2)[5-8]. Here, the potential of PLMDs to enhance RT and promote anti-tumor immunity was demonstrated.

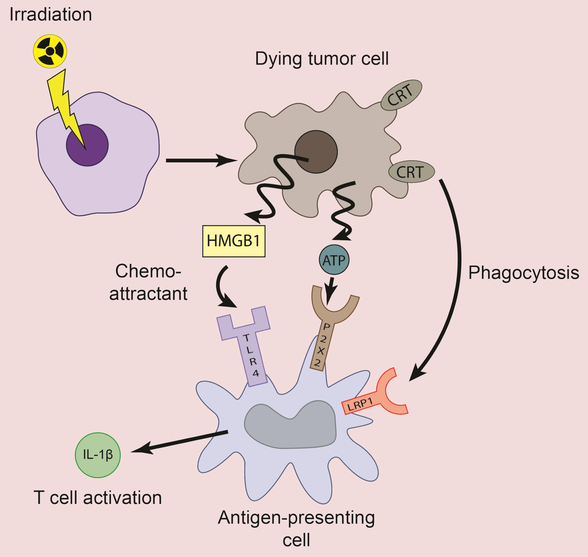


Fig 1. Schematic of radiation therapy (RT)-induced ICD and stimulation of adaptive immunity. Exposure of calreticulin (CRT), release of high mobility group box 1 (HMGB1) and secretion of ATP. These signals can stimulate phagocytosis of dying cancer cells by antigen-presenting cells, such as macrophages, allowing cross-presentation of tumor antigens to activate T cells.

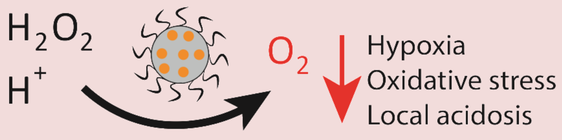


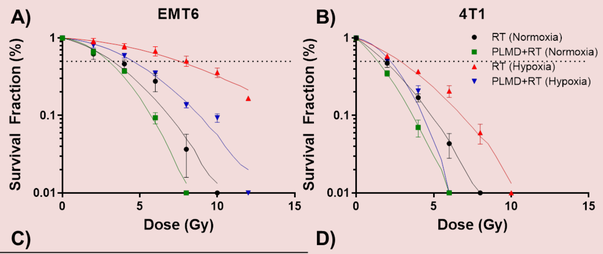
Fig 2. Reaction scheme of PLMD.

Methods

Murine breast cancer EMT6 and 4T1 cells conditioned under hypoxic conditions were subjected to irradiation of various doses with or without pre-treatment with PLMDs. Clonogenic assay was used to determine cell kill. A linear quadratic model was employed to evaluate radiation responses and radio-sensitization effect of PLMDs. ICD biomarkers, CRT, HMGB1 and ATP were assayed *in vitro*. Immune cell populations in orthotopic 4T1 tumor were analyzed by immunohistochemistry following treatment of tumor-bearing mice with PLMD and RT alone or in combination.

Results

PLMD enhances RT cytotoxicity *in vitro* against murine breast cancer cells under normoxic and hypoxic conditions



C)

EMT6 D50 (Gy)	Normoxia	Hypoxia
RT	3.25 ± 0.45	7.63 ± 0.18
PLMD+RT	2.99 ± 0.15	4.57 ± 0.27
% Decrease	8 %	40 %
4T1 D50 (Gy)	**Normoxia**	**Hypoxia**
RT	1.86 ± 0.16	2.86 ± 0.13
PLMD+RT	1.40 ± 0.11	2.22 ± 0.22
% Decrease	25 %	22%

D)

$$S = e^{-\alpha d - \beta d^2}$$

E)

EMT6	RT (Normoxia)	PLMD+RT (Normoxia)	% Change (PLMD+RT vs RT)	RT (Hypoxia)	PLMD+RT (Hypoxia)	% Change (PLMD+RT vs RT)
α	0.11 ± 0.06	0.04 ± 0.04	-175 %	0.02 ± 0.02	0.04 ± 0.03	+50 %
β	0.034 ± 0.005	0.06 ± 0.01	+43 %	0.009 ± 0.003	0.025 ± 0.003	+64 %
R^2	0.95	0.99		0.95	0.97	
4T1						
α	0.31 ± 0.05	0.41 ± 0.06	+24 %	0.17 ± 0.02	0.08 ± 0.08	-113 %
β	0.03 ± 0.01	0.06 ± 0.01	+50 %	0.024 ± 0.001	0.11 ± 0.01	+78 %
R^2	1.00	1.00		0.99	0.98	

Fig 3. Clonogenicity of a) EMT6 and b) 4T1 cells treated with 25 μM of PLMD and RT. c) D50 and percent (%) decrease fold-change are tabulated. d) The linear quadratic equation used to model the results. e) Linear-quadratic parameters α, β, and Pearson correlation coefficient are tabulated. Tabulated data are presented as mean ± S.D., n = 3.

Results

PLMD and RT increases calreticulin exposure, HMGB1 and ATP release in vitro in 4T1 cells

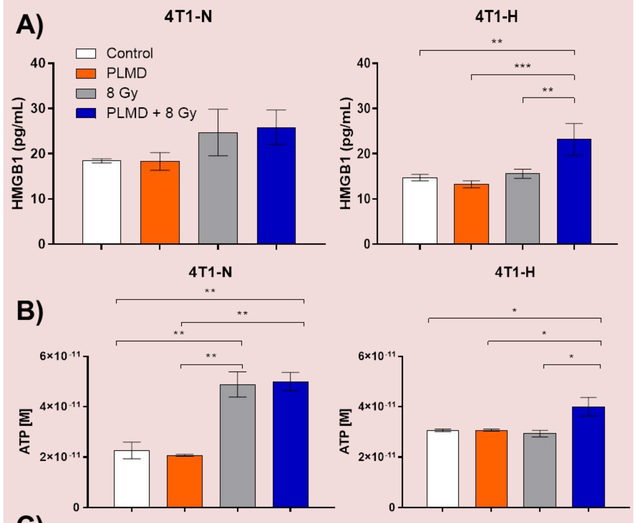


Fig 4. A) HMGB1 and B) ATP release induced by PLMD plus RT treatment in aerobic and hypoxic 4T1 cells. Cells were treated with PLMD (25 μM) followed by 8 Gy of irradiation within 30 min. HMGB1 and ATP was assayed at 48 h post-treatment. Bars are mean ± S.D., n = 3. Asterisk (*), (**), (***) and (****) represent p > 0.05, p > 0.005, p > 0.0005 and p > 0.00005 respectively. C) Representative confocal laser scanning microscope (CLSM) images of calreticulin (CRT) exposure in normoxic and hypoxic 4T1 cells treated with PLMD (25 μm) plus 8 Gy at 24 h post-treatment. DAPI (blue); CRT (green); n = 5; scale bar (white) is 20 μm.

Modulation of population of innate and adaptive immune cell with PLMD plus RT

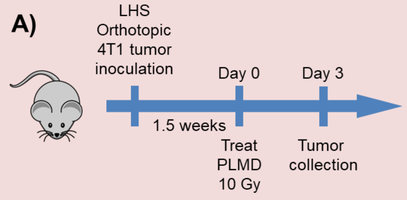


Fig 5. Modulation of innate and adaptive cell population with PLMD plus RT treatment at three days post-treatment. A) Experimental set up of the 4T1 model.

Results

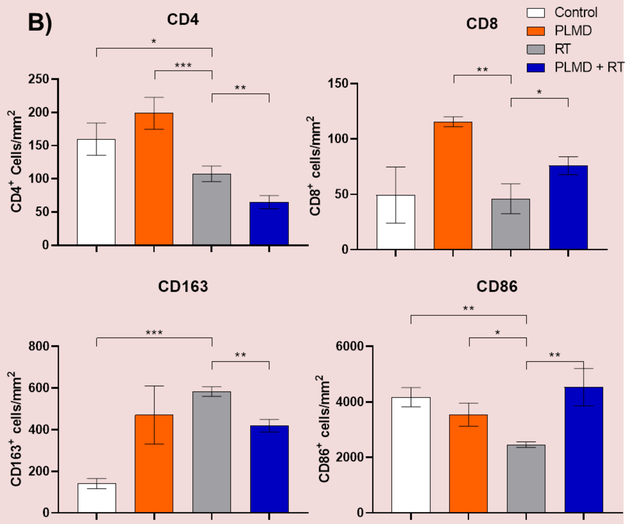


Fig 5. B) Quantification of whole tumor sections for $CD4^+$ T cell, $CD8^+$ T cell, $CD86^+$ macrophages and $CD163^+$ macrophages for all treatment groups. Bars are mean ± S.D., n = 3. Asterisk (*), (**) and (***) represent p > 0.05, p > 0.005 and p> 0.0005, respectively.

Conclusion

The results indicated enhanced anti-tumor immune response by PLMD+RT that significantly sensitized RT under hypoxia and amplified RT-induced anti-cancer immunogenicity.

Acknowledgements

CIHR Project grant & NSERC Discovery and Equipment Grants to XY Wu. UT Open Scholarship & OGS to Lip HY, Libya Scholarship to Zetrini A.

[1] Galluzzi L, *et al*. Immunogenic cell death in cancer and infectious disease. *Nature Reviews Immunology* 2017;**17**(2):97-111.
[2] Jing X, *et al.* Role of hypoxia in cancer therapy by regulating the tumor microenvironment. *Molecular cancer* 2019;**18**(1):1-15.
[3] Galanis A, *et al*. Reactive oxygen species and HIF-1 signalling in cancer. *Cancer letters* 2008;**266**(1):12-20.
[4] Vitale I, *et al.* Macrophages and metabolism in the tumor microenvironment. *Cell metabolism* 2019;**30**(1):36-50.
[5] Prasad P, *et al*. *Multifunctional albumin–MnO2 nanoparticles modulate solid tumor microenvironment by attenuating hypoxia, acidosis, vascular endothelial growth factor and enhance radiation response.* ACS nano, 2014. **8**(4): p. 3202.
[6] Gordijo CR, *et al*. *Design of Hybrid MnO_2-Polymer-Lipid Nanoparticles with Tunable Oxygen Generation Rates and Tumor Accumulation for Cancer Treatment.* Adv. Funct. Mater., 2015. **25**(12):1858.
[7] Abbasi AZ, *et al*. *Hybrid manganese dioxide nanoparticles potentiate radiation therapy by modulating tumor hypoxia.* Cancer Res., 2016. 76(22): 6643.
[8] Lip H,*et al*. *Redox-responsive nanoparticles enhance radiation therapy by altering multifaceted radio-resistance mechanisms in human castration-resistant prostate cancer cells and xenografts.* Radiother. Oncol., 2022.