The Granger-Causal Effects of Canady Helios Cold Plasma on the Inhibition of Breast Cancer Cell Proliferation

Annisa Elbedour [1], Xiaoqian Cheng [1], Saravana R.K. Murthy [1], Taisen Zhuang [1], Lawan Ly [1], Olivia Jones [1], Giacomo Basadonna [2], Michael Keidar [3], and Jerome Canady [1,3,4*]

1 Jerome Canady Research Institute for Advanced Biological and Technological Sciences, Takoma Park, MD 20912, USA
2. School of Medicine, University of Massachusetts, Worcester, MA 01605, USA
3 Department of Mechanical and Aerospace Engineering, The George Washington University, Washington, DC 20052, USA
Department of Surgery, Holy Cross Hospital, Silver Spring, MD 20910, USA
Email: drjcanady@jcri-abts.com

Introduction

Breast Cancer Classification

Breast cancer is a result of random mutations that allow breast cells to grow and proliferate without the tight restrictions imposed on them during the cell cycle. These mutations affect several of the cells' homeostatic parameters, including their cellular metabolism, proliferation rate, and the defenses they establish to circumvent cell cycle arrest or controlled death.

Table 1. Classification of Breast Cancer Cell Lines and Their Characteristics.

Classification	Immuno-profile	Ki-67 Level	Example Cell Line
Luminal A	ER+, PR+/-, HER2-	Low	MCF-7
Luminal B	ER+, PR+/-, HER2+	High	BT-474
HER2	ER-, PR-, HER2+	High	SK-BR-3
Triple-negative	ER-, PR-, HER2-	Low	MDA-MB-231

Cold Atmospheric Plasma

With its recent applications in oncology, regenerative medicine, and immunotherapy, CAP can be used for a myriad of different clinical treatments. When using CAP specifically for the treatment of tumors, it is known to elicit an oxidative response within malignant cancer cells, inducing cell cycle arrest and apoptosis.

Canady Helios Cold Plasma (CHCP) was developed at JCRI-ABTS (U.S. Patent No. 9.999,462-19, June 2018) and has been demonstrated to effectively eliminate various types of solid tumor including breast carcinoma. A phase I FDA investigational device exemption trial by CHCP to study the safety of the system was completed in April 2021.

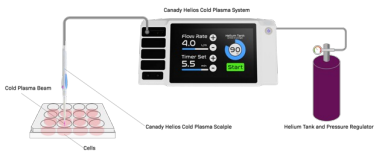

Figure 1. Schematic image of Canady Helios Cold Plasma setup for the treatment of breast cancer cells.

Granger Causality Model

Granger causality tests whether a variable, the potential "cause," is helpful for forecasting, or predicting, the behavior of another variable, the potential "effect." In a Granger model, each variable is regressed on all variables, including itself, at previous time points in order to reveal the temporal dynamics of a system across time. The outputted coefficients of regression (COR) are then analyzed to verify the usefulness of one variable to forecast another [1]. According to Granger causality, if X "Granger-causes" Y, then past values of X should contain information that helps predict Y above and beyond the information contained in past values of Y alone [2].

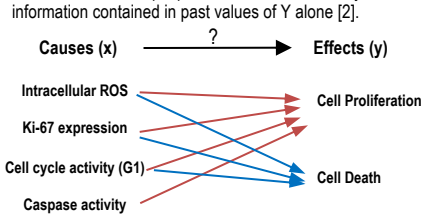

Figure 2. The Basis Behind Causal Testing.

In this study, Granger modeling was used to compare the individual effects of intracellular ROS, caspase activity, Ki-67 expression, and G1 phase activity on cell proliferation and cell death after 3- and 5-minute CHCP treatment for four different breast cancer cell-lines. These relationships were quantified through Granger causality's mathematical formulation using R statistical software.

CHCP-treated Breast Cancer Cells

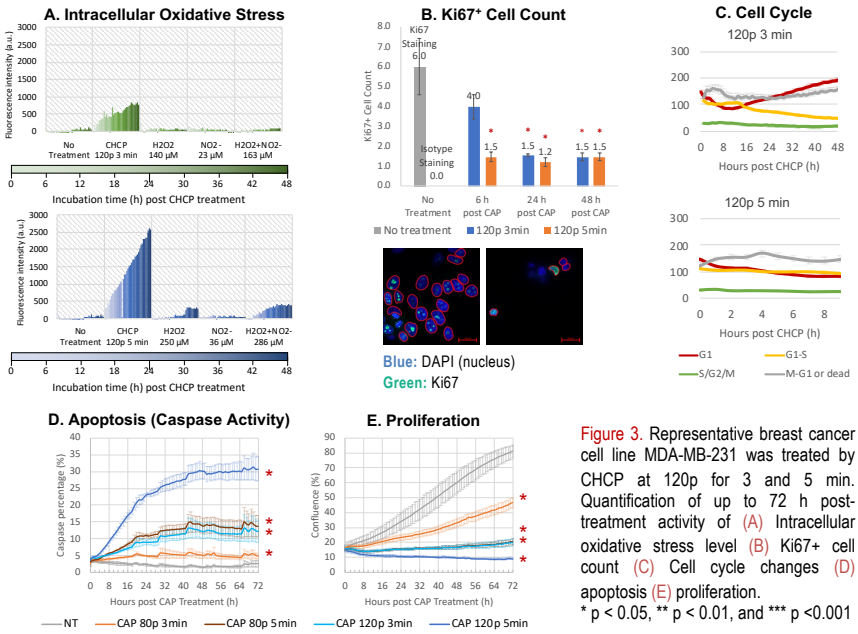

Figure 3. Representative breast cancer cell line MDA-MB-231 was treated by CHCP at 120p for 3 and 5 min. Quantification of up to 72 h post-treatment activity of (A) Intracellular oxidative stress level (B) Ki67+ cell count (C) Cell cycle changes (D) apoptosis (E) proliferation.
* p < 0.05, ** p < 0.01, and *** p <0.001

Granger-Causal Effects

3-minute CHCP treatment

Table 2. P values and coefficients of linear regression for all **cell proliferation** models for cells treated for 3 minutes (* p < 0.05). Only HER2+ cell lines BT-474 and SK-BR-3 displayed ROS Granger-causality.

Cell line	Result	Cause: ROS	Cause: Caspase	Cause: Ki-67	Cause: G1
BT-474	P value	$1.2x10^{-6*}$	0.0956	0.0206*	0.0003*
	COR	0.0029	0.0752	0.0364	0.0062
MCF-7	P value	0.2315	0.0009*	$< 2.2x10^{-16*}$	$1.4x10^{-13*}$
	COR	0.0061	2.3118	1.2369	0.047372
MDA-MB-231	P value	0.1305	$6.271x10^{-8*}$	0.2152	0.5003
	COR	0.0005	0.2057	0.0941	0.0025
SK-BR-3	P value	0.0006*	$8.94x10^{-10*}$	0.0111*	-
	COR	$3.2x10^{-4}$	0.0462	0.0615	-

5-minute CHCP treatment

Table 3. P values and coefficients of linear regression for all **cell proliferation** models for cells treated for 5 minutes. All variables were significant when treated for a longer period of time. The COR for the SK-BR-3 ROS model is significantly smaller than that of BT-474.

Cell line	Result	Cause: ROS	Cause: Caspase	Cause: Ki-67	Cause: G1
BT-474	P value	$1.8x10^{-9*}$	$1.1x10^{-10*}$	0.0061*	0.00121*
	COR	0.0009	0.0214	0.0707	0.0121
MCF-7	P value	0.0161*	$4.2x10^{-7*}$	0.008*	$1.19x10^{-12*}$
	COR	0.0012	0.1075	0.0623	0.0284
MDA-MB-231	P value	$4.5x10^{-6*}$	$1.2x10^{-8*}$	$< 2.2x10^{-16*}$	$< 2.2x10^{-16*}$
	COR	0.0007	0.0885	0.6924	0.0221
SK-BR-3	P value	0.0486*	$3.3x10^{-12*}$	$1.8x10^{-8*}$	-
	COR	$8.3x10^{-5}$	0.0511	0.0879	-

Table 4. P values and coefficients of linear regression for all **cell death** models for cells treated for 3 minutes (* p < 0.05). The results show that MDA-MB-231 model detected no causality for any variable.

Cell line	Result	Cause: ROS	Cause: Ki-67	Cause: G1
BT-474	P value	0.1823	0.08641*	0.03197*
	COR	0.001565	0.03311	0.003271
MCF-7	P value	0.001521*	0.0001978*	0.000331*
	COR	0.0012771	0.017391	0.0018635
MDA-MB-231	P value	0.09577	0.2085	0.5509
	COR	0.0004847	0.09009	0.002259
SK-BR-3	P value	$5.16x10^{-7*}$	0.000365*	-
	COR	$2.48x10^{-4}$	0.25092	-

Table 5. P values and coefficients of linear regression for all **cell death** models for cells treated for 5 minutes. ROS and Ki-67 showed causality for MDA-MB-231 under these conditions. However, G1 remained insignificant.

Cell line	Result	Cause: ROS	Cause: Ki-67	Cause: G1
BT-474	P value	0.01091*	$4.726x10^{-7*}$	0.4513
	COR	0.00223	0.26380	0.005350
MCF-7	P value	0.2384	0.1001	0.01122*
	COR	0.0007537	0.06361	0.023061
MDA-MB-231	P value	0.01123*	0.01146*	0.05247
	COR	0.0005037	0.23959	0.009367
SK-BR-3	P value	$5.98x10^{-5*}$	0.006272*	-
	COR	$2.46x10^{-4}$	0.55293	-

Conclusions

- Quantifying CHCP's relationship to intracellular ROS, caspase activity, Ki-67 expression, and cell cycle activity in the G1 phase elucidates the impact cold plasma has on disturbing different malignant cellular processes.
- Receptor status for each cell line greatly impacted the causal influence each variable had on cell proliferation and cell death.
- Computational modeling aids in understanding the systematic series of micro-cellular operations that occur with cancer treatment.

References

1 Granger, C.W.J., Investigating causal relations by econometric models and cross-spectral methods. Econometrica, 1969. 37:424–438
2 Siggiridou, E., Koutlis, C., Tsimpiris, A., & Kugiumtzis, D, Evaluation of Granger Causality Measures for Constructing Networks from Multivariate Time Series. Entropy 2019. 21(11),