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Role of Protein Kinase-C and Rho Kinase in the Cytotoxic Effects of Bitter Melon Extract on Metastatic Breast Cancer Cells

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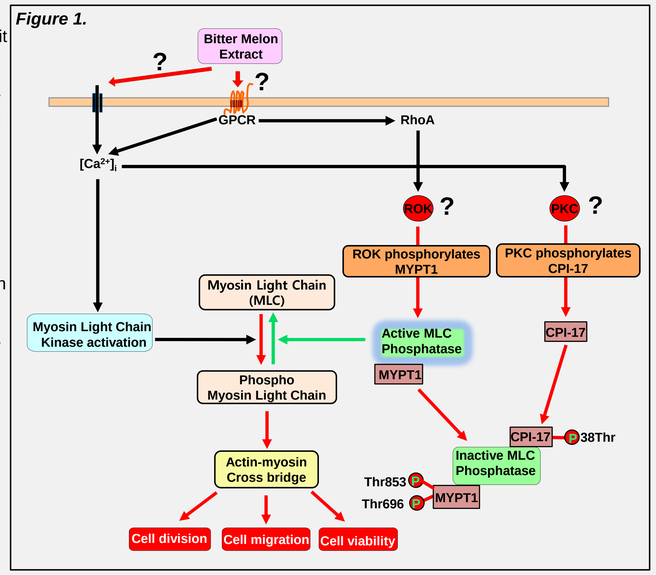
Introduction

Bitter melon is known to enhance uptake of glucose and lipid by healthy cells, and it is also known to inhibit growth of cancer cells [1,2,3]. However, the effects of bitter melon extract (BME) for inhibiting uncontrolled division of cancer cells depend on the types of cancer cells. Since breast cancer is one of the most common cancers in women [4], breast cancer cell line (MCF-7 cells) has been chosen in this project to investigate the effects of BME.

Rho Kinase (ROK) and Protein Kinase C (PKC) are critically involved in cell division, cell migration, and cell survival [5,6] (***Figure 1***). ROK phosphorylates MYPT1 [Myosin targeting subunit of myosin light chain phosphatase (MLCP)] and inhibits MLCP. PKC phosphorylates CPI-17 which then binds with MLCP and inhibits it. Thus, ROK and PKC both inhibit MLCP activity favoring balance tilt towards enhanced myosin light chain kinase (MLCK) activity which then phosphorylates myosin light chain (MLC). Phosphorylated MLC activates actin-myosin cross bridge formation which in turn regulates cellular processes such as cell migration, cell division, and cell survival.

However, roles of ROK and PKC inhibition on MCF-7 cells have not been established. In addition, whether the effects of BME are mediated by ROK or PKC are unknown. Thus, we aimed to investigate if BME exerts cytotoxic effects on breast cancer cells (MCF-7 cells) and if PKC and ROK mediate BME's effects.

Figure 1.



Experimental Procedures

1. Making bitter melon extract (BME)
Fresh bitter melons were purchased from an Asian grocery store. The y were washed and ground in a juice extractor. The juice extract was centrifuged at 5000 RPM/15 minute/9 times and filter sterilized. BME aliquots were stored at -80°C.

2. Dose-response effects of BME
Equal number of MCF-7 cells were plated in 250 mL culture flasks con taining DMEM medium and increasing amount of BME [0%, 0.5%, 1% , 2%, 5%, and 10% of BME (v/v)].

3. Testing roles of Rho kinase (ROK) and protein kinase C (PKC) inhibitors
To study whether PKC or ROK play any in mediating BME's effects, e qual number of MCF-7 cells were cultured in 100 mL culture flasks co ntaining DMEM medium in the following conditions; 0% BME, 1% BM E, PKC inhibitor (GF109203x; 0.5μM)/ ROK inhibitor (H-1152; 1 μM), and one of the inhibitors combined with 1% BME.

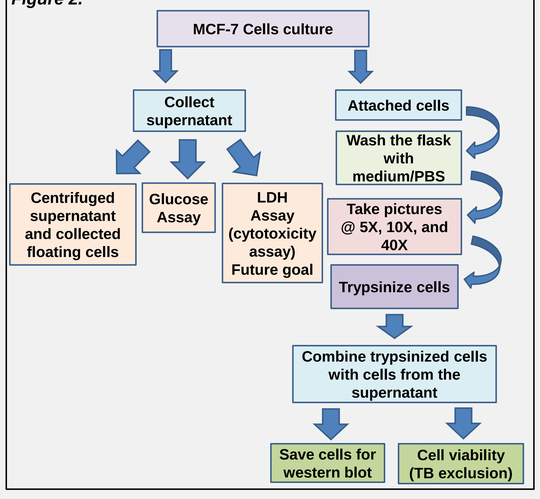
4. Taking pictures of cultures
After culturing cells for 6 days (for the dose-response study) and 3 da ys (for the inhibitor studies), pictures of cultures were taken at 5x, 10x, and 40x, and cell viability was determined using Trypan blue dye.

5. Glucose clearance from the medium
After culturing cells for a required number of days, culture was centrifuged, clear medium was obtained and remaining glucose in the DMEM medium was measured using a glucose monitoring system (Bioreactor Sciences).

6. Western Blot
After culturing cells for a required number of days, culture was centrifuged and cells were collected, protein was extracted using RIPA buffer and proteins candidates of the ROK pathways were detected using HRP-conjugated chemiluminescence system.

7. Statistical significance
Repeated t-tests were used to determine statistical significance for now. However, with anticipated increased number of observations, we plan to use multiple comparison ANOVA.

Figure 2.



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3. Ray RB, Raychoudhuri A, Steele R, Nerurkar P, 2010
4. Serban Comsa, Anca Maria Cimpean, and Marius Raica, 2015
5. AR Black and JD Black, 2013
6. Sandra Kumper, Faraz K Mardakheh, Afshan McCarthy, Maggie Yeo, et al, 2016

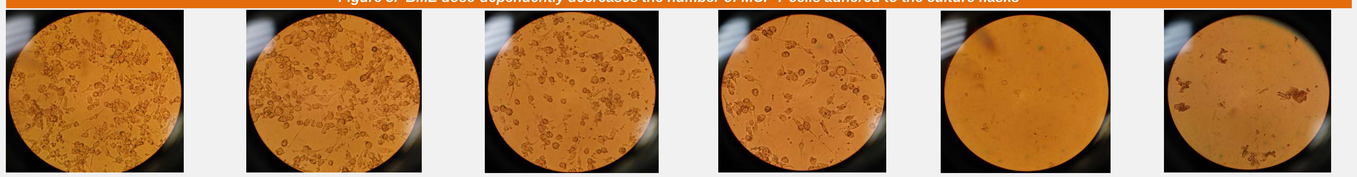
Acknowledgement

1. Marian University College of Osteopathic Medicine Biomedical Science Research Fund.
2. Thesis committee members (Drs. Michael Lafontaine, Bilon Khambu, and Medhane Cumbay)
3. Dr. Colleen Doci (Marian University) for providing MCF-7 cells

Results

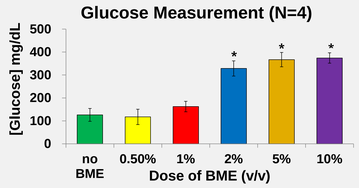
Bitter melon extract (BME) dose-dependently exerts cytotoxic effect on MCF-7 cells.

Figure 3. BME dose-dependently decreases the number of MCF-7 cells adhered to the culture flasks



Equal number of MCF-7 cells was cultured for 4 days in all the flasks and followed by washing in 1X PBS, pictures of cultures were taken at 40X magnification.

Figure 4. BME dose-dependently decreases glucose clearance from culture medium



After 4 days of culture, glucose in the medium was measured by glucose meter (Bio Reactor Sciences GF-100)

Figure 5. BME dose-dependently prevents drop in pH in culture medium suggesting less metabolism

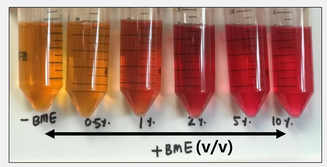
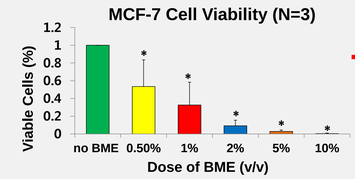
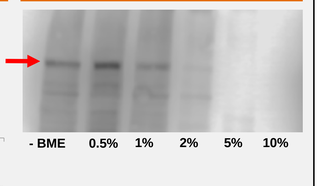


Figure 6. BME dose-dependently decreases viability of MCF-7 cells



Cell viability was determined with Trypan Blue dye using hemocytometer.

Figure 7. BME dose-dependently decreases MYPT1 expression



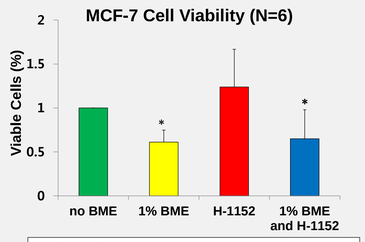
Western blot was done with sample size of one for three times.

Rho kinase (ROK) inhibition potentiates BME's cytotoxic effect on MCF-7 cells.

Figure 8. ROK inhibitor (H-1152, 1.0 μM) increases the number of MCF-7 cells adhered to the culture flask by itself and does not affect viability of MCF-7 cells when combined with bitter melon extract

Equal number of MCF-7 cells was cultured in all the flasks for 2 days followed by washing in 1X PBS, pictures of cultures were taken at 40X magnification.

Figure 9. ROK inhibitor (H-1152, 1.0 μM) seems to increase viability of MCF-7. The decreased cell viability from combined treatments is likely caused by bitter melon extract



Cell viability was determined with Trypan Blue dye using hemocytometer.

Figure 10. MYPT1 expression is decreased by BME and ROK inhibitor

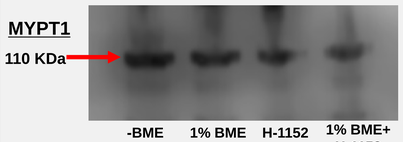
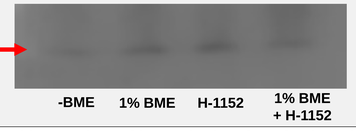


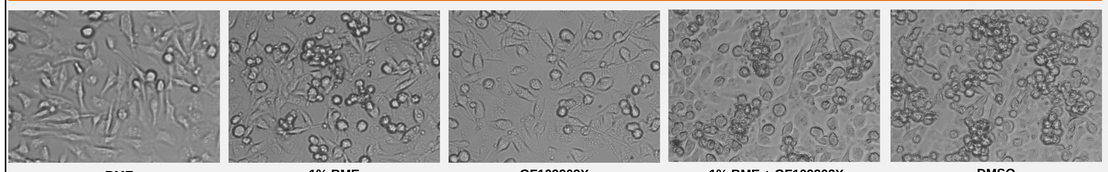
Figure 11. BME and ROK inhibitor increase MLC20 expression



Western blot was done with sample size of one for three times.

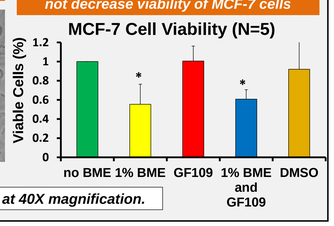
Inhibition of Protein kinase C (PKC) doesn't affect viability of MCF-7 cells.

Figure 12. PKC inhibitor (GF109203X, 0.5μM) does not affect number of MCF-7 cells adhered to the culture flask



Equal number of MCF-7 cells was cultured in all the flasks for 2 days and followed by washing in 1X PBS, pictures of cultures were taken at 40X magnification.

Figure 13. PKC inhibitor (GF109203X) does not decrease viability of MCF-7 cells



Conclusions

1. Bitter melon extract (BME) dose dependently exerts cytotoxic effect on MCF-7 cells .
2. BME decreases glucose clearance from the medium possibly due to less number of viable cells remaining in BME treated cultures.
3. ROK inhibitor, H-1152, seems to increase viability of MCF-7 cells (non-significant).
4. Protein kinase C does not affect viability of MCF-7 cells.

Future directions

1. Measure the changes in phosphorylation of ROK target protein, MYPT1, and that of PKC target protein, CPI-17, with bitter melon extract treatment.
2. Measure the change in phosphorylation of Myosin Light Chain with bitter melon extract treatment.
3. Measure cytotoxicity induced by bitter melon extract using Lactate Dehydrogenase (LDH) and MTT assays which are more specific than Trypan Blue exclusion assay that we performed.