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Cinnamon Extract Alters MCF7 Cell Morphology

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Abstract

Cancer prevention is one area of research that has gained interest in recent years. The anti-cancer effects of polyphenols and other nutraceuticals show great promise in prevention as well as therapeutics. In cancers that occur due to epigenetic modifications, molecules that provide these modifications are useful for both the prevention and reversal of disease. Our lab studies the effects of cinnamon extract on cell viability, growth and morphology. Cinnamon extract has high polyphenolic content and is believed to regulate a variety of transcription factors as well as epigenetic modifications. Our observations suggest that a significant change in morphology occurs in the MCF-7 breast cancer cells as a result of cinnamon treatment. Furthermore, these changes do not appear to be due to changes in cell adhesion. Epigenetic changes alter the expression level of genes that dictate cell morphology and therefore morphological changes suggest epigenetic modification are resulting from the cinnamon treatment. Cells were grown to 70-80% confluence and treated with either cinnamon extract or vehicle for 48-96 hours. Cells were screened for viability using both the trypan blue exclusion assay and the XTT assay. Our data demonstrate a change from polygimal to rounded morphology. This finding suggests that further research on gene expression levels should be completed.

Introduction

Our interest in this project stemmed from the knowledge that polyphenols have epigenetic effects. Epigenetic effects occur when there is a semi-permanent or permanent alteration of the transcription level of a given gene. In most cases this means the gene is either blocked due to methylation or the availability of the gene is limited due to changes in histone acetylation (figure 1). Numerous studies have shown epigenetic effects from the polyphenols in green tea as well as other dietary based phenols. Previous clinical studies in the lab have suggested epigenetic potential of cinnamon extract, making epigenetic effects on cancer cells particularly interesting. Further literature evaluation identified epigenetic effects from polyphenols on breast cancer cells, specifically the cell line we work with (MCF-7). Our goal was to explore this possibility by initially analyzing the DNA of treated cells for methylation. Upon treatment of the cells with the cinnamon extract and individual components, extraction of genomic DNA was difficult. This stimulated an evaluation of cell morphology in which we noted atypical structure for the MCF-7 cells treated with cinnamon extract and individual components. We hypothesized that this might be because the cells appeared to "ball up" as opposed to lying flat and adopting the polygonal appearance, thus we suspected cell adherence might to affected by treatment.

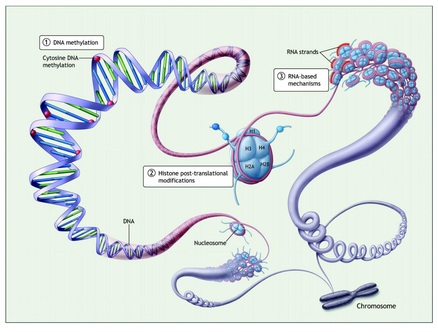

Figure 1. Summary of Epigenetic Effects on gene expression. Figure adopted from cicres.ahajournals.org

Methods

Monolayer Culture Methods. Monolayer cultures were expanded under the media conditions suggested by ATCC and varied as appropriate for experimental design.

Cell Viability Assay. All cultures were analyzed for viability using the trypan blue exclusion assay using an Olympus IM inverted microscope.

Photography. Cells were photographed using a Nikon Digital Sight DS-5M camera mounted on a Meiji Techno inverted microscope.

HPLC Analysis: The method used isocratic 50% acetonitrile/water, UV detection at 220 nm with an injection volume of 10 ul. Samples were run for 12 minutes using a C18 Reverse phase column (5 micron particle size, 0.5 mm id and 150 mm long).

Cell Adhesion Assay: Cells were grown under standard treatment conditions (DMEM plus 10% FBS with and without treatments (cinnamon extract, cinnamic acid, cinnamaldehyde, cinnamyl alcohol). Media plus treatment was removed via aspiration and cells were treated with trypsin until release was identified. Cells were centrifuged gently and washed 5 times with 1 X PBS. Cells were suspended in the media with various treatments, then plated in a 48 well array culture dish coated with one of the following Fibronectin, Collagen I, Collagen IV, Laminin I, Fibrinogen or BSA. Cells were allowed to adhere for 60 minutes. Treatment media was removed and cells were stained with the stain provided in the kit for 10 minutes. Stain was removed and cells were washed with deionized water 5 times. Lysing solution was then added and the cells were monitored at 560nm for optical density.

Preparation of Cinnamon Extract: Extract was prepared by grinding cinnamon in a mortar and pestle, allowing it to soak at room temperature overnight and then filtered via a Buchner funnel with filter aid.

Results

Figure 2. HPLC Analysis of Cinnamon Extract.

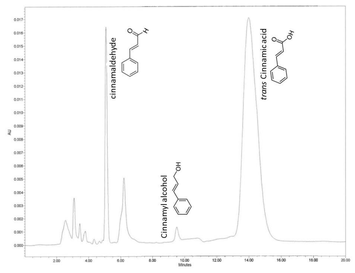

Figure 2. HPLC analysis of Cinnamon Extract. Peaks are assigned as shown. Our analysis of the cinnamon extract indicated two major components which we assigned as the acid and the aldehyde moieties. Additionally there were minor components with retention times between 2 and 5 minutes and one at 6.5 minutes. We are currently working on characterizing these peaks.

Figure 3. Comparison of MCF-7 cells.

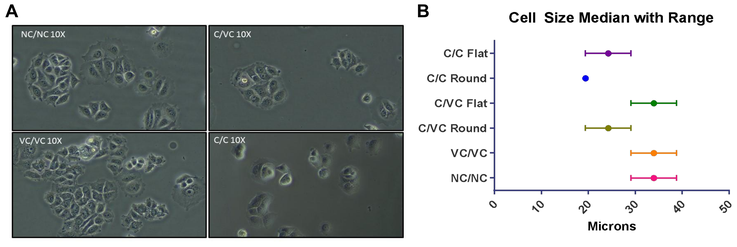

Figure 3. A. MCF-7 cells photographed for cell size and morphology analysis. All cells were plated, attached for 4 hours, and treated or fed. The media was removed at 24 hours and replaced with fresh media containing vehicle or 400 ug/ml cinnamon extract. The top left panel shows MCF-7 cells growth under standard media conditions continuously. The lower left panel shows cells treated with vehicle continuously. Cells treated with cinnamon extract following attachment, replaced with vehicle 24 hours later are shown on the top right panel. Cells in the bottom right panel were treated continuously with cinnamon extract. B. The graph at right shows the median cell size with full range of measurements. Cells treated with cinnamon extract continuously (C/C) were smaller in the round morphology. C/C cells that were flat were also smaller than VC/VC cells only.

Results

Figure 4. Adhesion Assay for MCF-7 Cells

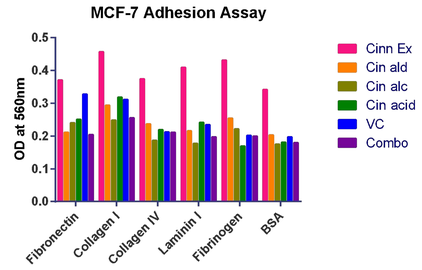

Figure 4. The adhesion assay was completed to determine if extract was interfering with adhesion via any one of these proteins. Based on the OD, all values are very low indicating that adherence does not occur well with any treatment. Standard values for BSA control are in the 0.1 range. As all of these values are close to the BSA control, preliminary analysis suggests adhesion is disrupted, however the VC is also disrupted. This data is therefore inconclusive and suggests that repetition is necessary.

Figure 5. Pictures of MCF-7 cells with various treatments.

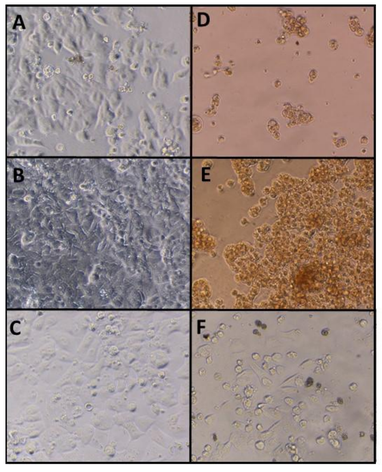

Figure 5. A. Vehicle Control B. Cinnamaldehyde (40µg/ml) C. Cinnamyl alcohol (40µg/ml) D. Cinnamic Acid (40µg/ml) E. Combination of Cinnamaldehyde, Cinnamyl alcohol, Cinnamic Acid (40µg/ml each) F. Cinnamon Extract with Cinnamaldehyde concentrations determined to be 40µg/ml. All photos were taken at 20X magnification. Cells in A,B and C have the typical polygonal morphology but are over confluent in panel B. The high concentration of cells in panel B may be forming some round structures because of over confluence. In panels D and E there is significant roundness and virtually no typical polygonal morphology. Panel E is over confluent which could cause rounding but these cells still have significant differences in appearance when compared to panel B. Panel F contains the extract treated cells where we do see some roundness and some polygonal structures.

Conclusions

In monolayer, treatment with cinnamic acid, and to a lesser extent cinnamon extract, disrupts the standard polygonal morphology seen with MCF-7 cells. This appearance is also observed in cells treated with the combination of all treatments. Although we hypothesize that the cinnamic acid is responsible for interfering with cell adhesion and causing the roundness of the cells, we can not yet confirm this with the adhesion assay as completed. Additional studies need to be completed to evaluate the morphological change. Cell culture needs to be grown for purposes of DNA extraction and or protein assay. At this point we have not been able to extract DNA from the round cells efficiently enough to analyze for methylation.

References and Acknowledgements

MCF-7 (ATCC® HTB-22™;PureLink ™ Genomic DNA Mini Kit by Invitrogen®; CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Colorimetric Format) by Cell Biolabs, Inc.; Takahashi K, Suzuki K. Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7 cells. Int. J. Cancer 55: 453-458, 1993. PubMed: 8375929; Paluszczak, J., V. Krajka-Kuzniak, and W. Baer-Dubowska. "The Effect of Plant Polyphenolic Compounds on the Proliferation and DNA Methylation in MCF-7 Cells." European Journal of Cancer Supplements 6.9 (2008): 150.