Targeting Chemotherapy Resistance in Non-Small Cell Lung Cancer via Berry Anthocyanidins

Stacy A. Henley1, Raghuram Kandimalla, Ph.D.2, Farrukh Aqil, Ph.D.2,3, Ramesh C. Gupta, Ph.D.1,2

Department of Pharmacology & Toxicology1, JG Brown Cancer Center2, and Department of Medicine3

University of Louisville, Louisville, KY 40202

Introduction

- Lung cancer is the leading cause of cancer deaths in the United States and the world.
- Multidrug resistance (MDR) is one of the main factors leading to the failure of chemotherapy, targeted therapy, and immunotherapy.
- Paclitaxel (PAC) is a leading chemotherapy for aggressive lung cancer. Drug resistance, specifically PAC resistance, thus plays an important role in the prevalence and mortality of lung cancer.
- The MDR gene encodes the membrane transporter multi-drug resistance protein (MDR-1), which works through an ATP dependent efflux mechanism. Lung cancer cells respond to cancer therapies, such as PAC, by overexpressing MDR-1 as a protection mechanism.
- Our lab has previously shown that native mixture of anthocyanidins (Anthos) isolated from blueberry, in combination with PAC, had significantly enhanced cell death in drug-sensitive lung cancer cells both in vitro and in vivo. The effects were even greater when Anthos was loaded onto milk exosomes (Aqil et al. Food & Function, 2017).
- Exosomes (Fig. 1), are endogenous nanovesicles that have been suggested as a potential drug carrier. Because of their size (30-100 nm), exosomes can readily enter cells and deliver their payloads. Exosomes could also be functionalyzed with tumor-recognizing ligands such as folic acid (FA) for targetability (Aqil et al. AAPS, 2017).
- There remains an urgent need to find a way to chemosensitize acquired resistance and potentially prevent resistance from occurring altogether in lung cancer therapy.
- In this study, we explored the use of the efficacious plant therapeutics (e.g., Anthos), in combination with chemo drugs (e.g., PAC), to enhance therapeutic response to overcome drug resistance in PAC-resistant lung cancer cells.
- We further evaluated the effect of Anthos and its exosomal formulation on the drug resistance protein (MDR-1) and if its downregulation could sensitize the drug-resistant cells to PAC.

Hypothesis

- We hypothesize that Anthos + PAC will promote cell death in PAC-resistant lung cancer cells.
- Furthermore, we hypothesize that the nano-formulation of Anthos will have greater efficacy at downregulating the MDR-1 protein compared to its free agent.

Results


Methods & Materials

Preparation of Exo-Anthos: Bovine milk-derived exosomes, Anthos and Exo-Anthos were gifts from Mr. Jeyaprakash Jeyabalan of 3P Biotechnologies, Inc. Briefly, exosomes were isolated from bovine milk through differential centrifugation and suspended in PBS. Native mixture of Anthos, isolated from bilberry, was dissolved in a mixture of acetone:toluene: ethanol (1:1:1 v/v). Anthos solution was then added to the exosomes by simple mixing. The mixture was kept at room temperature for 15 min, followed by low-speed centrifugation to remove unbound Anthos. Anthos-loaded exosomes were then collected by high-speed centrifugation.

Exosome Characterization: Atomic force microscopy (AFM) was used to determine surface characteristics and size of exosomes (AFM picture was provided by Mr. Jeyaprakash Jeyabalan of 3P Biotechnologies, Inc.).

Drug Loading Analysis: Anthos loading onto exosomes was determined by ethanol precipitation of exosomal proteins. The Anthos was measured by HPLC while the protein concentration was determined by BCA method spectrophotometrically. Percent Anthos loading was calculated by dividing the amount of Anthos with protein X 100.

Cell Culture: Cell line for PAC resistant lung cancer (TR-A549) was cultured in RPMI media with 10% (v/v) heat-inactivated fetal bovine serum and 1% antibiotics (penicillin/streptomycin), at 37°C in a humidified atmosphere of 5% CO₂.

MTT Assay: Cytotoxicity of Anthos against drug-sensitive (TS-A549) and drug-resistant lung cancer cells (TR-A549) was measured by MTT. After 24 h, samples lysates were measured using a microplate spectrophotometer at 570 nm. The data was expressed as percent inhibition versus a control.

Protein Concentration: Whole cell lysates were prepared using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Protein concentration of the cell lysates was measured using a BCA kit.

Exosomal Protein Concentration: Western Blot Analysis of exosomal proteins was performed using a 10% SDS-PAGE gel. The Protein bands were then transferred to a PVDF membrane and probed with antibodies specific for MDR-1, B-catenin, and GAPDH.

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References


Future Direction

- To further examine the influence of Anthos in detail on the MDR-1 mechanism.
- To evaluate the effect of Anthos + PAC on MDR-1 expression in drug-resistant lung cancer cells.
- To validate the drug-durable and possible chemosensitization effects of Anthos+Exo-Anthos through MDR-1 inhibition in drug-resistant lung cancer animal models.

Summary of Findings

- Exosomal formulation of Anthos showed significantly higher growth inhibition versus the free Anthos against A549 lung cancer cells; the exosomal delivery of Anthos increased the drug efficacy (a 20-fold reduction in IC₅₀, data not shown).
- Exosomes isolated by differential centrifugation process resulted in average particle size of about 75nm.
- The exosomes were characterized by i) AFM, and ii) presence of surface markers (CD63 and CD81) (not shown).
- Anthos loading efficacy was found to be about 7%.
- Drug-sensitive lung cancer responds well to PAC treatment with an IC₅₀ of 5.4 nM, whereas drug-resistant lung cancer has little or no response at all to PAC treatment.
- The Anthos enhanced the potency of PAC in drug-sensitive lung cancer cells when treated as combination.
- Anthos + PAC had a 20% decrease in IC₅₀ compared to Anthos alone in drug-resistant lung cancer cells.
- Both Anthos and Exo-Anthos showed a significant dose-dependent inhibition of MDR-1 in drug-resistant lung cancer cell line TR-A549. However, the effect was enhanced modestly with exosomal formulation.

Conclusions

- Both Anthos and Exo-Anthos appear to chemosensitize PAC resistant lung cancer cells through MDR-1 downregulation.
- Anthos+Exo-Anthos showed greater efficacy at lower doses, providing a non-toxic drug with tumor targetability.
- Clinical Impact: Because Exo-Anthos has shown to downregulate the MDR-1 protein at greater efficacy, it could be considered as an adjuvant therapy during cancer treatment to prevent or delay acquired resistance to chemotherapy drugs.