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3-Bromopyruvate and Genistein Combination Inhibits Glycolysis and Induces Cell Death in DU-145 and LNCaP Prostate Cancer Cells

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Abstract: Prostate cancer is among the leading cancer-related causes of death in United States. An estimated 161,360 new cases and 26,730 cancer related deaths are expected in 2017. Conventional thermotherapies flaws/short-comings include side effects which could be long-lasting and fatal. Current research is focused on attacking cancer cells by inhibiting signaling pathways in carcinogenesis, and finding molecular targets for potential therapeutic molecules. The long-term goal/objective of our study/project is to determine the efficacy of 3-Bromopyruvate (3BP)-Genistein (Gn) combination treatment to target glycolysis and induce cell death in LNCaP and DU-145 prostate cancer cells, at significantly lower concentrations while minimizing or eliminating potential side effects. Data from the preliminary studies revealed that: i) genistein significantly potentiates the treatment-induced apoptotic cell death of 3-bromopyruvate in both cancer cell lines; the mechanism of growth inhibition included targeting the energy metabolic pathways of the cells.

Key Words: - Prostate cancer cell lines; 3-bromopyruvate; Genistein isoflavone; 3BP-Gn combination therapy.

RESEARCH COMMUNICATION

Prostate cancer is one of the most common cancers found in American men, becoming the second leading cause of cancer related deaths in the United States of America. An estimated 161,360 new cases and 26,730 cancer related deaths are expected in the year of 2017 [1]. Conventional thermotherapy is flawed by induction of side effects which could be long-lasting and fatal. Developing alternative therapeutic treatments is an ongoing focus in research. Current researches are focusing on attacking cancer cells by inhibiting signaling pathways in carcinogenesis, inducing apoptosis molecules and growth inhibitors. The long-term goal/objective of our study/project is to determine the efficacy of 3-Bromopyruvate (3BP)-Genistein (Gn) combination treatment to target glycolysis and induce cell death in LNCaP and DU-145 prostate cancer cells, at significantly lower concentrations while minimizing or eliminating potential side effects.

In this study, LNCaP and DU-145 prostate cancer cells were incubated under humidified atmosphere at 37°C and CO_2 for 48 hr to achieve +80% confluence in 96-well microtiter plates (96 well-MTP). The cells were then exposed to varying concentrations of 3BP ($3BP_{60\text{-}160\ \mu M}$) and 3BP + genistein ($3BP_{60\text{-}160\ \mu M}$ + Gen_{60}), incubated for 48-72 hr and then analyzed/assayed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and Nitroblue tetrazolium (NBT) reagents. MTT assay was used to evaluate the cells' metabolic activity (treatment-induced cell death); Nitroblue tetrazolium assay (NBT) was used to assess treatment-induced intracellular ROS levels (and correlate this with cell death); and fluorescence microscopy was used to analyse and assess the kind/types of treatment-induced cell death (percentage apoptosis vs percentage necrosis).

The preliminary data revealed that: i) both treatment regimen (3BP and 3BP-Gn combination) induced cell death (apoptosis and necrosis) in both cancer cells; ii) treatment-induced cell death was concentration-dependent; the percentage cell death increased

concomitant with increasing concentration of both drug treatments (3BP and 3BP-Gn combination); iii) percentage cell death at each dosage level was significantly higher ($P < 0.001$) in the 3BP-Gn combination ($3BP_{60\text{-}100\ \mu M}$ + Gen_{60}) compared to the single 3BP treatment; iv) the NBT assay showed a dose-dependent decrease of ROS levels produced in both treatments while the 3BP-genistein combination treatment had higher levels of ROS induction compared to single 3BP treatment; v) treatment-induced apoptosis correlated with treatment-induced ROS levels.

In general, the data obtained in this study are in conformity with reports of previous studies which reported treatment-induced apoptotic cell death in cancer cells exposed to genistein or 3-bromo-pyruvate [2,3,4]. Unique aspects of cancer cells include their ability to alter their energy metabolism and evade cell death. These bioenergetic features allow the cancer cells to survive hypoxic conditions and enable their proliferation and invasiveness [5,6]. While normal cells produce most of their energy through mitochondrial respiration, cancer cells exhibit the "Warburg Effect", in which the cellular energy, adenosine triphosphate (ATP) production, is derived from aerobic glycolysis resulting in lactic acid production [3,7,8,9]. Furthermore, steady-state ROS balance is high in cancer cells versus normal cells, where increased persistent ROS may cause oxidative damage to DNA of cancer cells and initiation of apoptosis, suggesting that a delicate balance of intracellular ROS is required for cancer cell function [2, 3].

In cancer cells, high levels of ROS can result from increased metabolic activity, mitochondrial dysfunction, and increased activity of oxidases [2]. Consequently, targeting these signaling pathways via increasing intracellular ROS may reduce stimulation of glucose uptake and inhibit glycolysis. This can have many therapeutic implications such as depleting the cancer cell of bioenergy (ATP), inhibiting cell proliferation and consequent cell death. 3BP treated cells revealed 55% increased intracellular ROS production compared to control [10].

Genistein has been reported to have increased intracellular ROS at higher concentrations (>50 μM) [11]. Genistein isoflavone (genistein - 4',5,7-trihydroxyisoflavone) is a small molecule found in soy that has been found to possess potent anti-cancer activities [12]. In a study done by Gerhauser, genistein treatment of pancreatic cancer cells inhibited hexokinase, which is an important mediator of glycolysis [13].

In another study, Pavese discovered that genistein was able to decrease metastatic formation by inhibiting prostate cancer cell detachment and invasion [14,15]. These studies augment the potential significance of genistein in formulating treatment regimens for cancer prevention and/or treatment. Our data showed that genistein potentiates the anti-cancer activity of 3-bromopyruvate in a dose-dependent manner, indicating the potential therapeutic significance of the combination regimen. In-depth studies are in progress to delineate the signaling pathways and therapeutic targets/markers for the 3BP-Gn combination regimen in prostate cancer lines in vitro and in vivo.

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