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ABC-Mediated Multidrug Resistance Brought on by Therapy in PC-3 Prostate Cancer

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Abstract

Background: The number of deaths from prostate cancer is still high due to ATP Binding Cassette (ABC)-Mediated Multidrug Resistance (MDR). Overexpression of ABC transporters causes multidrug resistance in most prostate cancer chemotherapies. P-glycoprotein (P-gp) is one of the common drug transporters associated with MDR. There are no drugs approved by FDA to reverse MDR (inhibiting P-gp) in prostate cancer. This study utilized drug combination to reduce MDR expression by using 3-Bromopyruvate (3-BPA) to potentiate the therapeutic effect of SC-514. SC-514 is a relatively new hydrophobic dug, which has been shown to have anti-cancer effects via inhibition of NF-KB-dependent gene expression in cancer cells. 3-BPA is an alkylating agent, glycolytic inhibitor, and an anticancer drug that has a great potential to enhance the effects of anticancer drugs.

Aim: This study aimed to reduce acquired and intrinsic ABC-mediated multidrug resistance (MDR) by increasing the drug efficiency of SC-514 via drug combination with 3-BPA.

Method: Cell titer glow assay, multidrug resistance efflux assay, immunofluorescence assay and ELISA assay were utilized to investigate the drug efficiency of SC-514 in combination with 3-BPA and the number of drug resistance GR-PC-3 cells and PC-3 cells after treatment.

Results: Combination of SC-514 and 3-BPA significantly decreased intracellular ATP and the number of MDR cells in GR-PC-3 and PC-3 prostate cancer cells. SC-514 and/3-BPA treatments reduce NF-KB activation, IL-6 expression, and BCL2 expression. However, SC-514 and/3-BPA treatments increase the expression of Bax.

Conclusion: Combination of SC-514 and 3-BPA increased the therapeutic effect of SC-514 in prostate cancer treatment. The anticancer activities of SC-514 and 3-BPA in combination is promising for future drug development and drug combinations to completely reverse MDR in prostate cancer treatments.

Keywords: Multidrug resistance; SC-514; Drug combination; Toxicity; Potentiate; Prostate cancer

Introduction

Increased Incidence of Prostate Cancer

Prostate cancer (PCa) is the most common cancer in American men, after skin cancer [1]. The American Cancer Society's estimates for prostate cancer in the United States for 2020 are: About 1 man in 9 will be diagnosed with prostate cancer during his lifetime. Prostate cancer is more likely to develop in older men and in African-American men [2-4].

The type of cancer therapy will go a long way to determine prostate cancer progression or development. Most primary prostate tumor cells are initially sensitive to androgen deprivation therapy (ADT). However, despite the advances in PCa treatment resulting in reduction in mortality rates, and increased patient survival, PCa still remains the most common non-cutaneous malignancy [5].

Androgen deprivation treatment is very effective at inducing response for advanced or metastatic PCa [6-8]. However, more than half of those cases become resistant to androgen deprivation treatment after several years in what is termed castration resistant prostate cancer (CRPC) or hormone resistant (HR) prostate cancer [9]. Hormone resistant (HR) prostate cancer cells had a higher expression of IL-6, compared to murine prostate cancer cell line (TRAMP-C1 cells). Even though 81% of prostate cancers are pathologically organ-confined at time of diagnosis [10]. After diagnosis prostate cancer can metastasized to other organs of the body if the treatment is not effective. 3-BPA is a known chemotherapeutic drug by itself. But is has limitations in treating prostate cancer [11]. One of the ways to overcome limitations of 3-BPA is direct oxidation of NF-κB by Reactive Oxygen Species (ROS). The ROS produced inhibits DNA binding ability of NF-κB [12]. This is very crucial to prevent survival of prostate cancer because NF-KB is one of the major pathways utilized by prostate cancer for survival [13]. Cysteine, Cys-62 is in the Rel homology DNA-binding domain (RHD) and therefore its oxidation inhibits DNA binding [14]. ROS production by anti-cancer drug can impact this oxidation. SC-514 has been shown to be efficient in producing ROS [15, 16]. The ROS released by SC-514 has the potential to enhance the therapeutic effect of 3-BPA and vice versa leading to a synergistic effect between 3-BPA and SC-514. However, there is no assurance that the synergistic effect between 3-BPA and SC-514 is strong enough to reduce MDR in prostate cancer as result of metabolic reprogramming of prostate cancer cells [17].

Very few therapeutic approaches can disrupt metabolic reprogramming. This is because tumors usually consist of mixed

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populations of malignant cells, some of which seem to show drug-sensitivity, while others appear to be drug-resistant [18]. Chemotherapeutic drugs may kill drug-sensitive cells but leave behind a higher proportion of drug-resistance cells. In previous studies, great efforts have been made to overcome MDR, but only a limited degree of success was achieved in clinical applications [19]. Additionally, effective control of drug release rates can be extremely important for clinical practice, because specific drug release rates should be formulated to overcome specific disease conditions [20]. Particularly, the development of MDR of prostate cancer cells is known to be a complex multistep process. MDR in cancer cells occur at different stages and different mechanisms requiring different treatment concentrations and different drug exposure time [21]. Therefore, in order to achieve an efficient drug delivery system for PCa therapies, the drug release profile in tumors should be maintained at optimum therapeutic concentrations with minimum fluctuation.

Furthermore, MDR has been demonstrated to have a unique broad-spectrum resistance phenomenon [22, 23]. This broad-spectrum resistance was observed by overexpression of proteins such as the ABC transporters in tumor cells. ABC proteins antagonize drug activity [23]. The ABC transporters including P-glycoprotein, are located in the cell membrane, and are highly dependent on ATP for activity. Inhibition of glycolysis and consequent inactivation of the ABC transporters promote intracellular retention of anti-cancer agents, thus highlighting their cytotoxic effects on malignant cells.

The mechanisms underlying MDR are rather complex. One of the mechanism, called transporter-mediated efflux is a major component that has received enormous attention. The transporter mediated efflux is controlled by efflux transporters. These efflux transporters include P-glycoprotein (ABCB-1/P-gp) and multidrug resistance proteins (MRPs).P-glycoprotein mediated efflux is one of the main mechanisms for multidrug resistance in PCa that can support prostate proliferation, angiogenesis and metastasis.

Prostate cancer metastasis to the bone

Patients with advanced stages of prostate cancer, can have conditions in which the cancer cells spread to the bones. This condition is known as bone metastases. Bone metastases is an extremely common event in patients with advanced prostate cancer, particularly those with castration-resistant prostate cancer (CRPC). Bone metastasis commonly causes pain, increases the risk of fractures, and can lead to a life-threatening condition characterized by an increased amount of calcium in the blood called hypercalcemia. Treatments for bone complications may include drug therapy or radiation therapy. Despite the claim that 90% of adult cancer patients can be relieved of their pain, uncontrolled cancer-related bone pain is still a concern, particularly for patients living at home with metastatic bone disease. In fact, more than 90% of patients with metastatic prostate cancer have evidence of skeletal deformity and bone pain. This lead to increased rates of bone fracture in metastatic prostate cancer patients. Research proceedings from the Oncology Nursing Society indicates that there is no effective drug to alleviate the pain from bone cancer metastasis. This bone cancer metastasis occurs when prostate cancer was treated the first time. However, the treatment was not effective therefore leading to prostate recurrence in a form that is more aggressive and dangerous (Figure 1).

Prostate cancer recurrence

Prostate cancer recurrence may occur if ATP is made available to prostate cancer cells through any of the pathways indicated in fig 1 above. Like any other cancer, prostate cancer recurrence occurs when remission relapse. There are 3 types of recurrence (local, regional and distant). The type of recurrence depends on the location of the first tumor with respect to the final tumor location. The reasons and causes for the reoccurrence of prostate cancer is not fully elucidated. Some studies have observed a group of cancer cells. These cells are known

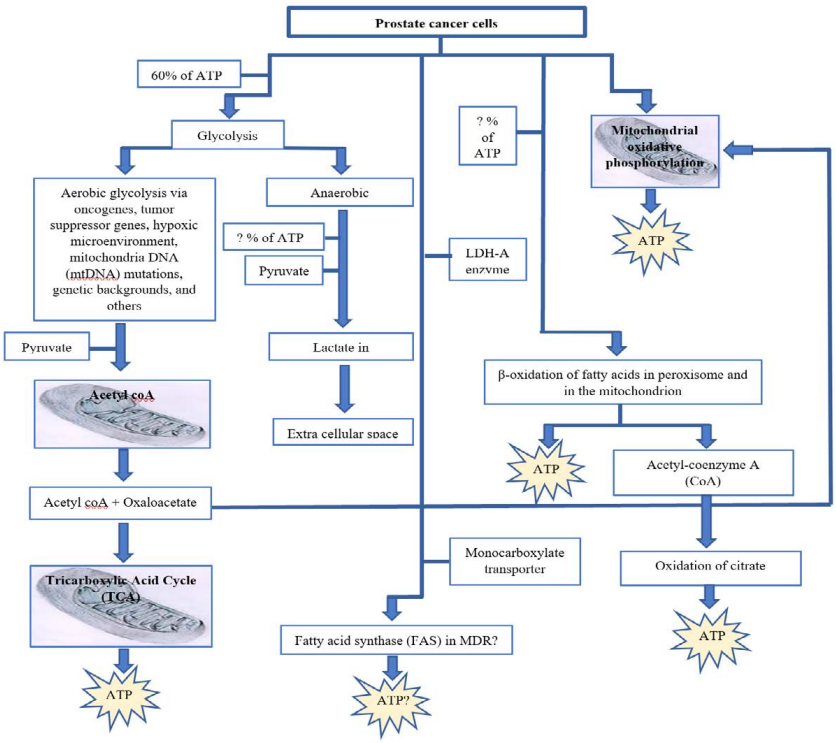

Figure 1: Energy production pathways in prostate cancer cells can impact the therapeutic efficiency of chemotherapeutic drugs

as cancer stem cells or cancer initiating cells. Cancer stem cells are potential cause of recurrence of prostate cancer due to their tumor-forming capability, self-renewal, and resistance to chemotherapy and radiotherapy. This recurrence may be linked to multidrug resistance in prostate cancer treatment.

Prostate cancer may relapse because of prostate cancer resistance to chemotherapeutic drugs. This condition is known as multidrug resistance (MDR). MDR has long been known as one of the challenges working against effective prostate cancer chemotherapy. In fact, the resistance to drugs by PCa cells is recognized as the primary cause of failure for chemotherapeutic treatments in prostate cancer. Growing evidence supports the idea that deregulated cellular metabolism is linked to such resistance. Indeed, both components of the glycolytic and mitochondrial pathways are involved in altered metabolism linked to chemo-resistance of prostate cancer. Multidrug resistance is characterized by resistance to a broad range of structurally and functionally unrelated chemotherapeutic drugs.

In another situation, multidrug resistance (MDR) occurs after long-term chemotherapy, resulting in refractory cancer and tumor recurrence. Therefore, combatting MDR is an important step in prostate cancer treatment.

Several mechanisms of resistance have been identified including altered levels of multidrug resistance associated protein (an efflux pump), topoisomerase II, and glutathione S transferase. Overexpression of the MDR-l gene product, P-glycoprotein (P-170), an integral plasma membrane protein involved in the active efflux of cytotoxic materials from the cell, is consistently associated with multidrug resistance in cultured cell lines selected for multidrug resistance and in certain tumors.

P-170 is expressed in many normal tissues including kidney, adrenal glands, large intestine, and liver indicating that it is involved in normal physiological functions including detoxification and transport of lipophilic molecules. Tumors arising from tissues that normally express P-170 may be intrinsically resistant to chemotherapeutic agents or, alternatively, tumors that were initially responsive to chemotherapy may develop multidrug resistance during the treatment regimen and subsequently not respond to therapy.

Multidrug resistance can be present at the time of diagnosis (intrinsic resistance) or can be acquired after initial treatment and remission of a cancer (acquired resistance). Although multiple mechanisms mediate multidrug resistance, the first mediator of multidrug resistance to be characterized at the molecular level was MDR1, also known as P-glycoprotein (Pgp) and ABCB1. The clinical importance of MDR1-mediated multidrug resistance has been best characterized in acute myelogenous leukemia. The role of MDR1 in solid tumors has been more difficult to discern, due to variations in methods of detection of MDR1 in tissues. Multiple efforts have been made to standardize methods for MDR1 detection using flow cytometry, immunohistochemistry and in situ hybridization. Most of these methods involve the use of monoclonal antibodies that are specific to MDR protein of interest. Several monoclonal antibodies have been produced to different epitopes of P-170 and subsequently used in studies of various human tumor types. Multidrug-resistant cells contain a plasma membrane efflux pump, the multidrug transporter, which actively expels certain hydrophobic drugs from the cytosol to the cell exterior. These drugs are usually positively charged at physiological pH. This efflux of positively charged molecules might deplete the cytosol of protons, raising the cytosolic pH. The cytosolic pH of multidrug-resistant cells directly using a pH-sensitive dye coupled to a membrane-impermeable molecule was examined. Multidrug resistance is characterized by cross-resistance of human tumors to several different chemotherapeutic agents to which the patient has not been previously exposed.

A recent report detailing a series of multi-institutional trials to assess sources of variability in assays to detect P-170 in tumor specimens recommended standardization of approaches to the detection of P-170 in clinical specimens, including careful control of sample fixation and antigen preservation.

The ABCB1 gene (previously MDR1), located at 7q21, encodes a membrane glycoprotein, which acts as an efflux pump and reduces intracellular drug concentrations. Gene copy number amplification is one of the chromosomal aberrations leading to the overexpression of the ABCB1 gene. It occurs intra-chromosomally, forming homogenously staining regions (HSR), or extra-chromosomally, forming double minutes (DM). Both types have been reported in ABCB1 regional amplifications in acquired drug-resistant cell lines from various cancers.

Although there are a considerable number of reports dealing with amplifications of the ABCB1 gene, little is known about the mechanisms underlying the amplification process. Our knowledge of the amplification process is very limited for amplification accompanied by other chromosomal rearrangements such as translocation, inversion, insertion, and deletion. One reason is complexity and heterogeneity of the rearrangements and another is lack of appropriate methods to monitor the specific chromosomal changes over time. PCa drug resistance may arise within PCa cells exploiting structures within the tumor micro-environment or stem cell niches to acquire invasive and survival advantages.

Intrinsic proliferation and survival pathways mediated drug resistance

Drug resistance mechanisms study in PCa, suggest that the alternatively-activated survival pathways may include activated receptor tyrosine kinases (RTKs) [6]. Moreover, epidermal growth factor (EGFR) and vascular endothelial growth factor receptor (VEGFR) are linked to signaling transduction pathways including Akt/PI3K or Ras/Raf/MEK/ERK pathways, which mediate cell proliferation and survival.

Treatment of prostate cancer with various agents targeting these and other pathways such as mammalian target of mTOR, MAPK/ERK VEGF, and its receptor VEGFR, have also been reported to be regulated by androgens in androgen-dependent tumors through activation of HIF1α. Androgen depletion leads to direct up-regulation of VEGF-C, which in turns activates AR coactivator BAG-1L expression that enhances AR transactivation. Activation of other receptors and their pathways, such as interleukin 6 (IL-6) or Wnt/β-catenin has also been reported to be involved in the crosstalk with AR. Similarly, Insulin-like growth factor 1 (IGF1) has also been reported to enhance AR function in low or absent androgen levels, and may promote the transition towards androgen-independence. Transforming growth factor β (TGFβ) was also reported to be overexpressed in PCa, and shown to exert diverse functions in stromal tumor cells **via** SMAD-dependent or SMAD-independent signaling pathways. Nuclear factor-kappa B (NFκB)/IL-6 and somatostatin receptor, have shown to either enhance or completely restore sensitivity to taxane-based therapy. These findings suggest that alternative signaling pathways may play a central role in drug resistance and provide valuable insight of overcoming the resistance by targeting these pathways.

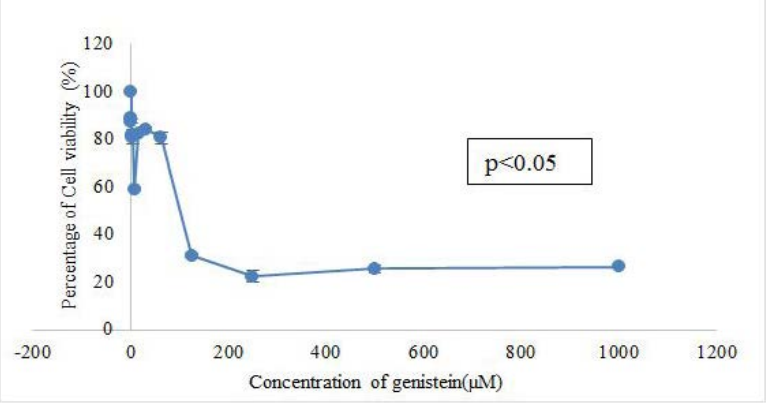

Figure 2: percentage cell viability of pc-3 cells treated with genistein.

However, once resistance to genistein is acquired, there are limited therapeutic options other than supportive care**.** Thus, it is critical to understand the mechanisms through which genistein-resistance develops in PCa. To mimic the clinical progression, we cultured and treat PC-3 cell lines with genistein, the cells that survived after 48hrs were labelled genistein-resistant prostate cancer cell lines.

Results

A good number of studies investigated the impact of genistein on prostate cancer (PCa) carcinogenesis. Our previous studies show that a subpopulation of prostate cancer cells survived after treating PC-3 prostate cancer cells with genistein in vitro. This study stepped up the concentration of genistein in PC-3 prostate cancer treatment in vitro to 1000μM (Figure 2). However, we observed that a subdivision of the prostate cancer cells still survived at 1000μM gensitein treatment. This subdivision of prostate cancer cells is called genistein resistant prostate cancer cells (GR-PC-3). In this study, we determine the impact of SC-514 and 3-BPA on genistein resistant PC-3 prostate cancer cells (GR-PC-3) and PC-3 prostate cancer cell lines (PC-3).

PC-3 prostate cancer cells were treated with increasing concentration of genistein (0.48μM - 1000μM). Data represented are the mean of ±SEM of three independent experiments. PC-3 prostate cancer cells that were viable after 48hrs of genistein treatment were labelled genistein resistant PC-3 prostate cancer cells in this study. These cells were incubated at 5% CO_2 and 37° C for another 48hrs. Cells were culture and treated (at their log phase, 48hrs culture and (80-90)% confluence) with 3-BPA and/or SC-514 in 96 well plates.

Before we investigated the number of MDR cells that survived after drug treatment, we utilized Cell titer glow assay to investigate the cell viability of the drug treated GR-PC-3 prostate cancer cells by quantifying amount of intracellular ATP in the cells. The result indicated that ATP level in form of luminiscence signal output decreases as drug concentrations increases. The combination treatment of 3-BPA and SC-514 consistently showed the lowest level of ATP or luminiscence signal output from 0.24μM -1000μM drug treatment.

Conclusion

The synergistic effect between 3-BPA and SC-514 was strong enough to reduce MDR in PC-3 and GR-PC-3 prostate cancer cell lines significantly. There was downregulation of multidrug resistant proteins and anti-apoptotic genes (NF-KB, IL-6, and BCL2) after treatment of GR-PC-3 and PC-3 prostate cancer cells with 3-BPA and/or SC-514. Apoptotic death in prostate cancer treatment with 3-BPA and SC-514 was elevated by increased expression of pro-apoptotic proteins (Bax) and decreased expression of anti-apoptotic proteins (NF-KB, IL-6 and BCL2).

3-BPA may be a good potentiator of other chemotherapeutic drugs with similar mechanism of action as SC-514. Combination drug treatments of other chemotherapeutic drugs may be equally or more effective in reducing the incidence of drug resistance and drug toxicities in prostate cancer treatments.

Acknowledgement:

Not applicable.

Conflict of Interest:

The authors declare no conflict of interest.

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