MOLECULAR ONCOLOGY 10 (2016) 949–965

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Combination therapy induces unfolded protein response and cytoskeletal rearrangement leading to mitochondrial apoptosis in prostate cancer

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ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
13 March 2016
Accepted 23 March 2016
Available online 31 March 2016

Keywords:
Prostate cancer
Anticancer drugs
Unfolded protein response
Combination therapy
Apoptosis
Mitochondria

ABSTRACT

Development of therapeutic resistance is responsible for most prostate cancer (PCa) related mortality. Resistance has been attributed to an acquired or selected cancer stem cell phenotype. Here we report the histone deacetylase inhibitor apicidin (APC) or ER stressor thapsigargin (TG) potentiate paclitaxel (TXL)-induced apoptosis in PCa cells and limit accumulation of cancer stem cells. TXL-induced responses were modulated in the presence of TG with increased accumulation of cells at G1-phase, rearrangement of the cytoskeleton, and changes in cytokine release. Cytoskeletal rearrangement was associated with modulation of the cytoplasmic and mitochondrial unfolded protein response leading to mitochondrial dysfunction and release of proapoptotic proteins from mitochondria. TXL in combination with APC or TG enhanced caspase activation. Importantly, TXL in combination with TG induced caspase activation and apoptosis in X-ray resistant LNCaP cells. Increased release of transforming growth factor-beta (TGF-β) was observed while phosphorylated β-catenin level was suppressed with TXL combination treatments. This was accompanied by a decrease in the $CD44^{+}CD133^{+}$ cancer stem cell-like population, suggesting treatment affects cancer stem cell properties. Taken together, combination treatment with TXL and either APC or TG induces efficient apoptosis in both proliferating and cancer stem cells, suggesting this therapeutic combination may overcome drug resistance and recurrence in PCa.

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http://dx.doi.org/10.1016/j.molonc.2016.03.007

1. Introduction

Prostate cancer (PCa) is the most diagnosed and second leading cause of cancer related death among American men (Siegel et al., 2013). Worldwide, PCa is the second most commonly diagnosed cancer and fifth leading cause of cancer related death among men (Ferlay et al., 2015). Metastasis is responsible for nearly all PCa mortality. First line therapy for metastatic PCa is androgen deprivation therapy (ADT). While beneficial responses are observed in most cases, recurrence is inevitable. Taxane-based chemotherapy is commonly used to treat men recurring from ADT, but this is not curative as resistant disease inevitably develops. Mechanisms of resistance to ADT fall into one of three general categories: restored androgen receptor (AR) signaling, bypass of AR signaling through use of other nuclear hormone receptors, or trans differentiation to a phenotype completely independent of AR signaling (Watson et al., 2015). Disease recurring in heavily treated patients exhibits several characteristics including resistance to apoptosis and increased drug efflux and/or metabolism, characteristics inherent in cancer stem cells (CSCs) or cancer-initiating cells (CICs) (Cojoc et al., 2015a; Fulda, 2013, 2015; Liu and Tang, 2011).

Paclitaxel (TXL) is a common prescription for the treatment of malignant epithelial cancers including PCa. TXL suppresses microtubule dynamics during mitosis thereby causing G2/M phase cell cycle arrest, growth inhibition and apoptosis (Yvon et al., 1999). The overexpression of multidrug transporters as well as hypoxia-inducing factor-1 in cancer cells diminishes the efficacy of TXL (Das et al., 2015; Statkiewicz et al., 2014). Other mechanisms underlying TXL resistance in PCa include changes in the kinetics of microtubule formation and elevated levels of antiapoptotic proteins like Bcl-2 (Murray et al., 2012; O'Neill et al., 2011).

Combination therapy is one of the key approaches to overcome drug resistance (Al-Lazikani et al., 2012). For example, TXL has been used in combination with other anticancer drugs like butyrate, bevacizumab, and the Akt inhibitor MK-2206 to treat different types of cancer (Hata et al., 2014; Molife et al., 2014; Rivkin et al., 2014). In advanced and progressive PCa, TXL in combination with estramustine or carboplatin showed increased antitumor activity (Kelly et al., 2001, 2003). Synergistic therapeutic efficacy of TXL was observed in combination with KML001 (sodium meta-arsenite) in treatment resistant PCa (Zhang et al., 2012). In advanced, hormone-refractory PCa, combinations of TXL, carboplatin, etoposide, and estramustine have shown enhanced antitumor activity in preclinical studies (Smith et al., 2003). Despite improved responses, no currently used single or combination therapy is curative in patients with metastatic PCa.

In order to overcome therapeutic resistance in the treatment of PCa, several unconventional compounds like apicidin (APC) and thapsigargin (TG) have been evaluated as potential anticancer drugs. APC is a cyclic tetra-peptide, which causes histone deacetylases (HDAC) inhibition, increases accumulation of cells at G1 phase in a dose-dependent manner, and blocks cell migration and invasion of cancer cells (Ahn et al., 2009, 2012). TG is the active ingredient in several chemotherapeutic pro-drug formulations that induce ionositol-3-phosphate (IP3)-independent intracellular calcium (Ca^{2+}) release and apoptosis by disrupting intracellular free Ca^{2+} levels (Dubois et al., 2013). TG also causes cancer cells to accumulate in G1 phase (Beaver and Waring, 1996). Considering TXL, APC, and TG function through distinct mechanisms of action (mitotic inhibitor, HDAC inhibitor, and ER stressor, respectively), combination therapy using these drugs may provide new options for overcoming therapeutic resistance in the treatment of PCa.

We hypothesize that resistance of metastatic PCa cells to TXL can be inhibited by abrogation of TXL-induced G2/M arrest since dividing cells are more sensitive to death (Mitchison, 2012; Valeriote and van Putten, 1975). Here, we observe that TG reverses cell cycle arrest induced by TXL leading to alterations in the cytoskeleton and mitochondria. Combining TXL with TG or APC caused cell death mainly through the mitochondrial pathway of apoptosis. Furthermore, TXL in combination with TG or APC reduces the level of CSCs or CICs, that are typically chemoresistant. These findings suggest that these combinations may effectively target CSCs/CICs with potential implications for treating patients suffering from recurrent, therapeutically resistant PCa.

2. Materials and methods

2.1. Cells and reagents

Androgen-dependent (LNCaP) and androgen-independent cell lines (DU145 and PPC1) were procured from American Type Culture Collection (ATCC, USA). E006AA and its highly tumorigenic derivative E006AA-hT (Koochekpour et al., 2004, 2014) were kindly provided by Dr. Koochekpour at Roswell Park Cancer Institute. X-ray irradiated cells were generated by Dr. Anna Dubrovska and were culture as described earlier (Cojoc et al., 2015b). Cells were maintained in RPMI-1640 medium, DMEM, and McCoy's 5A medium supplemented with 2 mM L-glutamine, 10% FBS (Atlanta Biologicals, USA), and 1% penicillin and streptomycin. All cells were cultured at 37 °C in a humidified atmosphere in the presence of 5% CO_2. All human cell lines were authenticated using the Short Tandem Repeat (STR) DNA profiling every 6 months.

Antibodies for p53, p21, α-tubulin, E-cadherin were procured from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibodies for cyclin B1, Bcl-xL, Bcl-2 and caspases were obtained from BD Biosciences (San Jose, CA, USA). Antibody for actin was purchased from MP Biomedicals, LLC. Antibodies for Bax and Bak were obtained from Upstate (Billerica, MA, USA). Heat shock protein 60 (Hsp60), Hsp70, Hsp90, Hsp10, and phospho-β catenin (T41/TS45) were obtained from MP Scientific and Cell Signaling, respectively.

2.2. Cell cycle analysis

Cell cycle analysis was carried out using propidium iodide (PI) staining according to the methods described earlier (Fried et al., 1976). In brief, treated or untreated cells were harvested