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Perifosine induces differentiation and cell death in prostate cancer cells

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Abstract

We analyzed the mechanism of action for perifosine (D-21266), a new synthetic alkylphospholipid Akt inhibitor, using LNCaP and PC-3 prostate cancer cells. Perifosine treatment of PC-3 cells resulted in cytostatic and cytotoxic effects. Cytostatic effects were characterized by cell growth arrest, cell cycle block, and morphological changes, such as a cell enlargement and granulation, hallmarks of differentiating PC-3 cells. Specific differentiation markers including prostasomal, secretory and plasma membrane proteins, and keratins were induced by perifosine. Among them, we detected strong induction and secretion of CEACAM5 protein. In contrast, perifosine strongly reduced caveolin-1 RNA levels. Cytotoxic effects included para-apoptosis, apoptosis, and necrosis. To pursue the mechanisms responsible for these activities we focused on signaling pathways that lie downstream of Akt. Perifosine-triggered GSK-3β activation in PC-3 and LNCaP cells resulted in the expression of GSK-3β-related differentiation markers. This expression was reduced in the presence of specific siRNA for GSK-3β or for its target CREB protein. The use of the GSK-3β inhibitor lithium chloride indicated that GSK-3β partially protects prostate cancer cells from the cytotoxic effects of perifosine. Together, these findings indicate that perifosine induces GSK-3β-related differentiation and caspase-independent cell death in prostate cancer PC-3 cells. In addition our results identify specific biomarkers for perifosine therapy.
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Keywords: Perifosine; Prostate cancer; PC-3; LNCaP

1. Introduction

Prostate cancer remains the most commonly diagnosed malignancy in men and second only to lung cancer as a leading cause of tumor deaths in males [1]. Currently there is no cure for locally advanced or metastatic prostate cancer. Prostate cancer progression to an androgen-insensitive state

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is associated with the development of resistance to cell death. Therefore, it is essential to understand the molecular pathways responsible for the survival of androgen-independent prostate cancer cells. This understanding will enable development of effective, targeted therapies for this disease.

Recent studies indicate that phosphatidylinositol 3-kinase (PI3K)-Akt pathway plays an important role in regulating cell proliferation and cell survival in many malignances including prostate cancer [2]. The kinase activity of Akt is stimulated by a variety of extracellular stimuli, such as growth factors, cytokines, chemokines, integrin engagement and T-cell receptor. We previously demonstrated that caveolin-1 overexpression leads to stimulation of Akt through the inhibition of serine/threonine protein phosphatase PP1 and PP2A [3]. The activation of Akt leads to the phosphorylation and regulation of a wide spectrum of its substrates involved in multiple cellular processes such as cell survival, cell growth, cell differentiation, cell cycle progression, cell proliferation, and cellular metabolism [4].

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an important negative regulator of Akt activation as it antagonizes the actions of PI3K. However, PTEN is mutated or deleted in a substantial number of cancers including prostate, breast, ovarian cancers and glioblastomas. Cells with PTEN mutation or deletion often exhibit a constitutive PI3K activity [2].

Perifosine (D-21266), a new synthetic alkylphospholipid, was shown to be a potent Akt inhibitor in prostate cancer PC-3 cells [5] and activator of the stress-activated protein kinase/c-jun-NH2-kinase (JNK) pathways in human leukemia cells [6].

GSK-3β is a characterized physiological substrate of Akt [7]. GSK-3β is an evolutionary conserved, ubiquitous serine/threonine kinase whose activity is inhibited by Akt phosphorylation on Ser^{9} in response to growth factor stimulation. There is evidence of the diverse functions of GSK-3β including regulation of cellular activity, structure, and survival [8]. GSK-3β is a critical negative regulator of both PI3K and Wnt cell signaling [9,10]. The ability of GSK-3β to phosphorylate the regulatory domain of β-catenin, a ligand-dependent co-activator of androgen receptor, places it in the category of a tumor suppressor gene. Activated GSK-3β was also shown to phopshorylate CREB on Ser^{129} in prostate cancer PC-3 cells [11]. Phosphorylated CREB on Ser^{129} and Ser^{133} was also found in the nuclei of low grade (well differentiated) prostate cancer but decreased in high grade (poorly differentiated) prostate cancer.

In the present study, we demonstrated that perifosine treatment of PC-3 cells resulted in the induction of differentiation which was characterized by morphologic changes, and induction of genes coding for prostasomal, secretory proteins, and keratins among others. We demonstrated that perifosine-induced Akt inhibition results in the activation of GSK-3β and its translocation to the nucleus of differentiating cells. We demonstrated that perifosine activates the GSK-3β/CREB pathway in both PC-3 and LNCaP cells. We also demonstrated that GSK-3β protects differentiated cells from cell death and contributes to the expression of multiple differentiation markers.

2. Material and methods

2.1. Cell cultures and chemicals

LNCaP and PC-3 cells were obtained from ATCC (American Type Culture Collection, Manassas, VA). Cells were cultured in 5% CO_2 at 37 °C in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin (Invitrogen). Perifosine (AOI Pharmaceuticals, Memphis, TN) was dissolved in PBS to prepare a fresh 10 μM stock solution. To determine cell numbers, PC-3 cells were plated in 60 mm plates at 3×10^5 per plate or in 100 mm plates at $1–2 \times 10^6$ per plate a day prior treatment. Attached cells were harvested with trypsin and counted using a Beckman Coulter cell counter (Fullerton, CA). To obtain photographs of control and treated cells, cells were washed three times with PBS and stained with a Giemsa solution (Sigma, St. Louis, MO) for 1 min. Images of stained cells were captured with a conventional Zeiss inverted microscope using transmitted light and phase contrast imaging. Cell viability was measured using an MTS assay according to the manufacturer's protocol (Promega, Madison, WI) or by means of flow cytometry using calcein-AM (Sigma) staining. For calcein-AM staining, concentration of harvested LNCaP and PC-3 cells was adjusted to 1×10^5 cells/ml. Cells at 1×10^5 were incubated with 100 nM calcein-AM for 15 min and analyzed by flow cytometry using Beckman Coulter Epics XL (Beckman). Usually 10,000 cells were analyzed in the appropriate channel. Percentages of viable control cells were considered to be 100% for re-calculation of viability of treated cells. Cell cycle analysis was performed as described previously [12]. Annexin V/propidium iodide (Sigma) staining was performed as described previously [13] and analyzed by flow cytometry.