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Cancer Detection and Prevention 32 (2009) 319–328

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Differential effects of cholesterol and phytosterols on cell proliferation, apoptosis and expression of a prostate specific gene in prostate cancer cell lines

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Accepted 20 December 2008

Abstract

Background: The purpose of our study was to show the apoptotic and anti-proliferative effects of phytosterols as distinct from cholesterol effects on prostate cancer cell lines, and also their differential expression of caveolin-1, and a prostate specific gene, PCGEM1. *Methods*: PC-3 and DU145 cells were treated with sterols (cholesterol and phytosterols) for 48 h, followed by trypan blue dye exclusion measurement of cytotoxicity and MTT cell proliferation assays, respectively. Cell cycle analysis was carried out microscopically, and by propidium iodide uptake using flow cytometry. Sterol induction of oncogenic gene expression was evaluated by RT-PCR. Apoptotic cells were identified by immunocytochemistry using DNA fragmentation method, and by annexin V adhesion using flow cytometry. *Results*: Physiological doses (16 μM) of these sterols were not cytotoxic in these cells. Cholesterol-enrichment promoted mitosis (54 and 61% by microscopy; 40.8 and 34.08% by FACS analysis in PC-3 and DU145, respectively) and cell growth ($P < 0.05$), while phytosterols suppressed mitosis (29 and 35% by microscopy; 27.71 and 17.37% by FACS analysis in PC-3 and DU145, respectively), and significantly induced tumor-suppression ($P < 0.05$) and apoptosis. We demonstrated for the first time that cholesterols upregulated the expression of PCGEM1 even in androgen-insensitive prostate cancer cell lines. Phytosterols reversed this effect, while upregulating the expression of caveolin-1, a known mediator of androgen-dependent proto-oncogene signals that presumably control growth and anti-apoptosis. *Conclusions*: Phytosterol inhibition of PCGEM1 and cell growth and the overexpression of caveolin-1, suggests that poor disease prognosis anchors on the ability of caveolin-1 to regulate downstream oncogene(s) and apoptosis genes. Sterol intake may contribute to the disparity in incidence of prostate cancer, and elucidation of the mechanism for modulation of growth and apoptosis signaling may reveal potential targets for cancer prevention and/or chemotherapeutic intervention. Sterol regulation of PCGEM1 expression suggests its potential as biomarker for prediction of neoplasms that would be responsive to chemoprevention by phytosterols.
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Keywords: Prostate cancer; Caveolin-1; Sterols; Cholesterol; Phytosterols; PCGEM1; Apoptosis; Proto-oncogene; Biomarkers; Chemoprevention

1. Introduction

High prevalence and poor prognosis of prostate cancer has been associated with increased intake of saturated fats and cholesterol [1,2]. To the contrary, dietary phytosterols, which are plant cholesterol counterparts, have reportedly suppres-

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0361-090X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.cdp.2008.12.002

sive effects on cell growth [3,4]. The contribution of high sterol diets to the etiology, progression or prevention/reversal of prostate cancer may be understood from cholesterol's crucial role in membrane organization, dynamics, function and sorting [5]. Associated with membrane cholesterol is the protein caveolin-1 (cav-1), a vital component of caveolae, which are cytomorphologically pitted vesicular membrane invaginations organized as specialized lipid rafts [6,7]. Basically, caveolae is formed by the homo- and hetero-oligomerization of the three currently known caveolins. Of these caveolins, cav-1 is involved in signal transduction largely because of the presence of a 20 amino acid micro-domain which can bind a variety of signal proteins [6], most often leading to downstream signaling events [8,9]. cav-1 has been identified as a marker of aggressive prostate carcinoma [10,11] that promotes progression to the metastatic phenotype [12]. The significance of cav-1 in cell signaling is demonstrated by its coordinating the interaction, redistribution and co-localization of several proteins with each other during experimental clustering of raft components [13]. Typically, cav-1 co-localizes with androgen receptors within lipid raft domains to mediate androgen-dependent signals [14,15], especially downstream signals that modulate the expression of genes implicated in unregulated cell growth [6].

Unregulated cell growth occurs mostly when cells loose their ability to undergo apoptosis, often leading to cancer. Various studies have confirmed the loss in cell growth control following alteration of apoptotic pathways [16]. The susceptibility of a cell to apoptosis is widely believed to be mediated by the expression of a complex family of genes that principally include p53 [16], which also mediate the transcriptional activation of cav-1 [17]. Thus, cholesterol-enrichment which results in oxidative stress [18], often lead to DNA damage and increase in p53 protein expression [19], G1 cell cycle arrest and interaction of numerous pro- and anti-apoptosis proteins [16]. Cholesterol-mediated dysregulation of cav-1 [10], and apoptotic suppressor genes [16] by aberrant p53 highlights the significance of p53 in cholesterol-mediated cell growth, and anti-apoptosis. In all, it is likely that understanding the mechanism of cholesterol-mediated transcription of apoptosis suppressors may be significant in elucidating the contribution of sterols in the etiology, progression or prevention/regression of prostate cancer.

Overall, the importance of sterols in the promotion of cell growth may therefore, depend on their orchestrating the co-localization of cav-1 and androgen receptor at the plasma membrane [14,15]. Androgen receptors elaborate the biological effects of androgens in target cells, by mediating the transcriptional regulation of androgen-regulated genes [20]. Recent data suggest ethnic variation in the pattern of expression of a prostate specific androgen-regulated gene (PCGEM1) [21], which promote unregulated cell growth. Although this proto-oncogene is irregularly expressed in high risk and poorly progressed prostate cancer [21], its transcriptional regulation by sterols and cav-1 to our knowledge has not been elucidated. Based on evidence that cav-1 is differentially transcribed by either sterol status [13,22] resulting in modulation of cell growth, we hypothesized that these sterols regulate prostate cell growth by promoting or repressing the transcription of cav-1 and its downstream signals. To test this hypothesis, we examined the effects of different sterol-enrichment on cav-1 expression, cell growth, apoptosis, and on the expression of downstream proto-oncogene PCGEM1, using PC-3 and DU145 prostate cancer cell lines.

2. Materials and methods

2.1. Cell culture

Androgen-independent prostate cancer cell lines PC-3 and DU145 were obtained from ATCC (Manassas, VA). The cells were cultured in MEM with 10% FBS, 1% penicillin/streptomycin, 1% glutamine, 1% non-essential amino acids, 0.1% gentamicine and fungizone and buffered with 0.75% HEPES at 37 °C in 5% CO_2 for 24 h. The medium was then changed to 1% FBS-MEM, and the cells incubated for 24 h before treatment with sterols. Experimental media were supplemented with sterols (cholesterol or phytosterols; 10% campesterol: 75% β-sitosterol) (Acros organics, NJ) at final concentrations of 16 μM. The phytosterol combination chosen represent the typical percentage concentration of β-sitosterol (78–83%) relative to phytosterols found in peanuts; which is a reported classic phytosterol source [23]. Optimum sterol concentration of 16 μM was chosen after dose–response experiments produced results that were consistent with physiological levels for phytosterols, and with previously observed values [3,24]. For all treatments, sterols were mixed in the media with a sterol carrier (2-hydroxypropyl)-β-cyclodextrin (β-CD) (Sigma–Aldrich, St. Louis, MO) and supplied to the cells as complexes. To provide the sterols in an assimilable and nontoxic form, the sterol to cyclodextrin molar ratio was maintained at 1:300 (16 μM sterol: 5 mM β-CD) as previously reported [24,25]. All treatments were performed in triplicates. After stimulation for 48 h, the cells or total RNA were analyzed in different experiments.

2.2. Measurement of sterol effects on cell viability

PC-3 and DU145 cells (5000 cells/cm^2) were seeded in triplicates into 6-well plates for 24 h. The medium was replaced with 1% FBS-MEM for 24 h, then the cells were supplemented with 16 μM sterols and vehicle for 48 h and 72 h. Viability of cells given different sterol treatment was measured by trypan blue dye exclusion method. After 48 h, 1×10^6 ml^{-1} cell suspension for viability assay was prepared by trypsinization, centrifugation and counting with a hemocytometer. A 1:1 dilution of 200 μl of the cell suspension was made using 0.4% trypan blue solution and