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Theaflavins induced apoptosis of LNCaP cells is mediated through induction of p53, down-regulation of NF-kappa B and mitogen-activated protein kinases pathways

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Abstract

Prostate cancer (PCA), the most frequently diagnosed malignancy in men, represents an excellent candidate disease for chemoprevention studies because of its particularly long latency period, high rate of mortality and morbidity. Infusion of black tea and its polyphenolic constituents have been shown to possess antineoplastic effects in androgen dependent PCA in both in vivo and in vitro models including transgenic animals. In the present study, we report that black tea polyphenol, Theaflavins (TF)-induced apoptosis in human prostate carcinoma, LNCaP cells is mediated via modulation of two related pathways: up-regulation of p53 and down-regulation of NF-kappa B activity, causing a change in the ratio of pro- and antiapoptotic proteins leading to apoptosis. The altered expression of Bcl-2 family member proteins triggered the release of cytochrome-*C* and activation of initiator capsase 9 followed by activation of effector caspase 3. Furthermore, TF also affected the protein expression of mitogen activated protein kinases (MAPK) pathways. Our results demonstrated that TF treatment resulted in down-regulation of phospho-extracellular signal-regulated protein kinase (Erk1/2) and phospho-p38 MAPK expressions. We conclude that TF induces apoptosis in LNCaP cells by shifting the balance between pro-and antiapoptotic proteins and down-regulation of cell survival pathways leading to apoptosis. Further extending this work, we also showed that TF induces apoptosis in androgen independent PCA cell line, PC-3 through caspases and MAPKs mediated pathways. Thus, effect of TF on PCA cell lines seems to be irrespective of their androgen status.

Keywords: Theaflavins; LNCaP Cells; Apoptosis; Prostate cancer

Introduction

Prostate cancer (PCA) is the most common malignancy and the second leading cause of male death in USA and many European countries. About 10% of men in the USA develop clinically detectable PCA in their lifetime (Greenlee et al., 2000). The incidence of clinically relevant PCA is much lower in Asian countries, although the incidence of microscopic or latent prostate carcinoma does not differ substantially (Fair et al., 1997). Average annual cancer incidence rates in India ranged from 5.0 to 9.1 per 100,000/year (Hebert et al., 2006). In India about 85% of prostate cancers are detected at late (III and IV) stage in contrast to the US where 15% of cancers are diagnosed at late stage (Hebert et al., 2006). These observations have led to the hypothesis that environmental factors and life style, such as diet are capable of altering the progression of this disease. Understanding the mechanisms involved in the progression of PCA from a latent to a clinically relevant form, and discovering compounds affecting these pathways will be helpful in the management of PCA (Klein and Fischer, 2002). The use of dietary supplements and plant-derived products has shown a great promise, owing in part to some encouraging pre-clinical and clinical observations. In vitro mechanistic experiments are considered essential preludes and requisites to more lengthy and costly animal and human studies (Li et al., 2005). Black tea, derived from the plant *Camellia sinensis*, is most popular beverage after water all over the world. Studies conducted on cell-culture systems and animal models reported

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that the black tea polyphenols provide protection against a variety of cancer types (Banerjee et al., 2005; Chandra Mohan et al., 2005; Mukhtar and Ahmad, 2000; Larsson and Wolk, 2005; Siddiqui et al., 2006; Wu et al., 2003; Yang et al., 2002; Zhou et al., 2003). Studies also suggest that these black tea polyphenols could be developed as potential therapeutic agents (Bickers and Athar, 2000; Kelloff et al., 2000).

Several epigenetic alterations leading to constitutively active mitogenic and cell survival signaling, as well as loss of apoptotic response, are involved in uncontrolled growth of PCA, leading to androgen-independent growth, apoptosis resistance, and increased expression and secretion of angiogenic factors (Kish et al., 2001). Therefore, one targeted approach for PCA prevention, growth control, and/or treatment could be the inhibition of molecular events involved in PCA growth, progression and apoptosis resistance. Earlier polyphenolic fraction isolated from green tea, at a human achievable dose (equivalent to six cups of green tea per day), was shown to inhibit PCA development and metastasis in transgenic adenocarcinoma of mouse prostate (TRAMP) model that closely mimics progressive form of human prostatic disease (Gupta et al., 2001). In vitro studies have shown that (−)-epigallocatechin-3-gallate (EGCG), the major polyphenolic constituent of green tea, causes cell cycle arrest and apoptosis of androgen-responsive human prostate carcinoma LNCaP as well as androgen-unresponsive human prostate carcinoma DU145 cells (Ahmad et al., 1997; Gupta et al., 2004). EGCG had a concurrent effect on two important transcription factors p53 and NF-kappa B, causing a change in the ratio of pro-and antiapoptotic proteins namely Bax/Bcl-2 leading to apoptosis (Hastak et al., 2003). Tumor suppressor gene *p53*, the guardian of the genome, is the most frequently altered tumor suppressor in human malignancies with more than 50% of solid tumors including prostate cancer due to deletion or point mutation or by other mechanisms, such as increased MDM2 expression, increased Raf or Akt signaling (Agarwal et al., 1998). Therefore, compounds capable of modulating these events will have the potential to prevent PCA. Another potential mechanism involves the activation of anti-apoptotic genes like *bcl-2* that compete with the proapoptotic pathways activated by p53 (Hastak et al., 2003). p53 is also responsible for cell cycle arrest upon DNA damage and is also a key regulator of apoptosis (Vousden and Lu, 2003). In contrast to the role of p53 as a negative regulator of growth signals, the NF-kappa B family of transcription factor, initiate cell survival pathways. The importance of NF-kappa B members in modulating cellular growth, apoptosis and development is well documented (Verma, 2004). Immuno histochemistry of human prostatectomy specimens' demonstrated over-expression of the active subunit of NF-kappa B, p65 at early stages of PCA (Sweeney et al., 2004). Since both NF-kappa B and p53 are activated in response to a variety of stimuli, it is conceivable that these transcription factors works antagonistically. Mitogen activated protein Kinase (MAPK) cascades are among the most thoroughly studied of signal transduction systems, and have been shown to participate in a diverse array of cellular programs including cell differentiation, cell movement, cell division and cell death (Ray et al., 2006). Extracellular signal regulated protein kinase (Erk) 1 and 2, one of the MAPKs family members, have been shown to be constitutively active in human PCA and functionally relevant in vitro (Siddiqui et al., 2004). The polyphenolic antioxidants of black tea have been shown to possess chemopreventive potential against PCA (Siddiqui et al., 2004). A complete understanding of molecular targets for PCA in combination with chemopreventive effects of tea polyphenols may be useful in developing novel approaches for its prevention and treatment.

In the present study we demonstrated the role of p53 and NF-kappa B in black tea polyphenol, theaflavins (TF) mediated apoptosis in LNCaP cells. We report that TF treatment causes induction of p53 and inhibition of NF-kappa B activation. We further demonstrated activation of p53-dependent downstream target Bax with concomitant down-regulation of Bcl2 and MAPKs pathways leading to apoptosis. Further extending this work we showed that TF also induces apoptosis in androgen insensitive cell line, PC-3.

Materials and methods

Materials

The human PCA cell lines LNCaP and PC-3 were obtained from National Centre for Cell Science Pune, India. LNCaP and PC-3 cells were maintained in RPMI 1640 and DMEM/F12 (1:1) respectively supplemented with 10% fetal bovine serum, glutamine, and antibiotics at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. All the chemicals of culture grade were procured from Gibco, Life Sciences, India. Purified TF (>98% pure) and antibody specific for β-actin (clone AC-74) were purchased from Sigma (St Louis, USA). The mouse monoclonal phosho-p38 MAPK, phospho Erk1/2, phospho-JNK1, pro and active caspase 3, pro and active caspase 9, cyt-C, NF-kappa B antibodies having cross reactivity with human samples were procured from Cell Signaling Technology (Beverly, Massachusetts). Anti-p53 mouse monoclonal antibody specific for wild type protein (clone PAb 1620), Bcl-2 rabbit polyclonal IgG and Bax rabbit polyclonal IgG antibody were procured from Oncogene Research Products (Cambridge, USA). The rabbit antimouse horseradish peroxidase or goat anti rabbit horseradish peroxidase conjugate secondary antibodies were obtained from Bangalore Genei (Bangalore, India). The nitrocellulose membranes were obtained from Sartorious, (Goettingen, Germany). ERK inhibitor (PD98059), p38 inhibitor (SB203580), and JNK inhibitor (SP600125) were obtained from Calbiochem (CA, U.S.A.) and dissolved in 0.05% DMSO. Pan caspase inhibitor (Z-VAD-fmk), caspase 3 inhibitor (Z-DEVD-fmk) and caspase 9 inhibitor (Z-LEHD-fmk) were purchased from Enzyme Systems (CA, U.S.A.) and dissolved in 0.05% DMSO. Rests of the chemicals were of analytical grade purity and were procured locally.

Treatment of cells

TF dissolved in phosphate buffered saline (PBS) was employed for the treatment of cells. For dose-and time-dependent studies, the