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Caffeic acid phenethyl ester (CAPE) prevents transformation of human cells by arsenite (As) and suppresses growth of As-transformed cells

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Abstract

Recent evidence suggests that inflammatory cytokines and growth factors contribute to arsenite (As)-induced human carcinogenesis. We investigated the expression of inflammatory cytokine mRNAs during the transformation process induced by chronic As exposure in non-tumorigenic human osteogenic sarcoma (N-HOS) cells using gene arrays, and results were confirmed by RT-PCR and protein arrays. Caffeic acid phenethyl ester (CAPE), a naturally occurring immunomodulating agent, was used to evaluate the role of inflammatory factors in the process of As-mediated N-HOS cell transformation and in As-transformed HOS (AsT-HOS) cells. We found that an 8-week continuous exposure of N-HOS to 0.3 μM arsenite resulted in HOS cell transformation. That exposure also caused substantial decreases in inflammatory cytokine mRNAs, such as interleukin (IL) IL-1α, IL-2, IL-8, IL-18, MCP-1, TGF-β2, and TNF-α, while it increased c-jun mRNA in a time-dependent manner. Co-incubation of N-HOS with As and CAPE (0.5–2.5 μM) prevented As-mediated declines in cytokine mRNAs in the co-treated cells, as well as their transformation to anchorage independence, while it caused decreases in c-jun mRNA. CAPE (up to 10 μM) had no effect on growth of N-HOS cells. However, CAPE (1–10 μM) treatment of AsT-HOS cells inhibited cell growth, induced cell cycle G2/M arrest, and triggered apoptosis, accompanied by changes in cytokine gene expression, as well as decreases in cyclin B1 and cdc2 abundance. Resveratrol (RV) and (−)•epigallocatechin gallate (EGCG), preventive agents present in grapes and green

Abbreviations: α-MEM, minimum essential medium alpha; As, arsenic; AsT-HOS, arsenic-transformed human osteogenic sarcoma cells; CAPE, caffeic acid phenethyl ester; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; EGCG, (−)•epigallocatechin gallate; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte-macrophage colony stimulating factor; HOS, human osteogenic sarcoma cells; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MIF, macrophage migration inhibitory factor; N-HOS, non-transformed parental human osteogenic sarcoma cells; RV, resveratrol; RT-PCR, reverse transcription-polymerase chain reaction; TGF, transforming growth factor; TNF, tumor necrosis factor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

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tea, respectively, induced similar changes in AsT-HOS cell growth but required much higher doses than CAPE to cause 50% growth arrest (<2.5 μM CAPE versus 25 μM RV or 50 μM EGCG). Overall, our findings suggest that inflammatory cytokines play an important role in the suppressive effects of CAPE on As-induced cell transformation and in the selective cytotoxicity of CAPE to As-transformed HOS cells.

Keywords: Apoptosis; Cdc2; Cyclin B1; Inflammatory cytokines; EGCG; G2/M interphase; Cell growth arrest; Gene and protein arrays; Prevention; Resveratrol

1. Introduction

Arsenic, one of the most common environmental contaminants, is a well-known human carcinogen (IARC, 1980). Millions of people in many countries are consuming drinking water contaminated by arsenic (in the form of arsenite and arsenate), and about 1.5 million industrial workers are potentially exposed to arsenic and its derivatives in the working environment (IARC, 1980; NIOSH, 1984; Stohrer, 2001). Many epidemiological studies have shown that arsenic exposure causes cancers in the human skin, urinary bladder, lung, and probably other organs (IARC, 1980; NIOSH, 1984; NRC, 1999). However, the molecular mechanisms of how arsenic causes human cancers remain elusive. Some investigators thought arsenic does not likely act through the classic genotoxic and mutagenic mechanisms as it is generally agreed that arsenic is a very poor mutagen and only at toxic concentrations [reviewed in (Basu et al., 2001; Rossman, 2003)]. It does cause an unusual "delayed" mutagenesis after many generations (Mure et al., 2003). Recently, animal tests showed that arsenic in a form of dimethylarsinic acid (DMA, a major metabolite of arsenic in most mammals including humans) acts as a complete carcinogen since it did induce bladder cancer in F344 rats (Wei et al., 2002). Dr. Waalkes' group found that arsenic also is a complete transplacental carcinogen in mice (Waalkes et al., 2004). However, the animal models for inorganic arsenic carcinogenesis have been limited (Kitchin, 2001; Rossman et al., 2001a, 2002). As a result, studies on the mechanisms of arsenic carcinogenicity have been carried out mainly in cell transformation models, especially in murine cells because human cells in culture have been difficult to transform in vitro (Rhim, 1993). Human osteogenic sarcoma (HOS) cell line is generally non-tumorigenic in nude mice; this characteristic and its low saturation density have made it useful in cell transformation studies (Sidhu et al., 1991; Rani et al., 1993; Rhim, 1993; Vihinen et al., 1996). Chronic exposure to low doses of arsenite proved capable of transforming HOS cells, but the molecular and cellular mechanisms by which arsenite transforms human cells are still unknown (Rossman et al., 2001b).

Many studies have demonstrated that arsenite is capable of triggering oxidative stress by a variety of pathways [reviewed in (Hughes, 2002; Rossman, 2003)]. Arsenite also shares many properties with tumor promoters by affecting cellular signaling and changing expression of genes involved in cell proliferation and transformation (Cavigelli et al., 1996; Germolec et al., 1998; Hamilton et al., 1998; Huang et al., 1999; Hughes, 2002). Arsenite can act as a co-carcinogen with UV light in a mouse skin model (Rossman et al., 2001a). Chemicals possessing tumor initiating and/or enhancing properties, such as 7,12-dimethyl benz[*a*]anthracene (DMBA), 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and UV radiation commonly induce chronic inflammation and oxidative stress (Wei and Frenkel, 1993; Frenkel et al., 1995; Soriani et al., 1999). It is thought that inflammatory reactions, characterized by the modulation of various cytokines and other inflammatory factors, may play an important role during the process of carcinogenesis [reviewed in (Frenkel, 1992; Shacter and Weitzman, 2002)]. For example, our previous study showed that topical exposure of SENCAR mice to DMBA initially triggered a significant increase in mouse skin interleukin (IL)-1α, a pro-inflammatory cytokine that initiates a cascade of other cytokines and growth factors (Li et al., 2002). This study also found that the induction of IL-1α contributes to malignancy, as anti-IL-1α antibodies significantly decreased skin carcinoma volume in mice exposed to DMBA in comparison to the volume of carcinomas arising in mice that were treated