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<b>13. ABSTRACT (Maximum 200 Words)</b> In this study we investigated the effects of these two dietary RXR ligands (agonists) on the cell growth and retinoid metabolism of cultured human prostate cancer cell lines. Retinoic acid (RA) treatment for 72 hours and 120 hours did not have a significant effect on the growth of PC-3 cells. However, RA did suppress the proliferation of LNCaP cells, especially after 120 hours. The specific RXR agonist BMS 188649 inhibited cell proliferation in both cell lines. Meanwhile, both phytanic acid and DHA inhibited the growth of PC-3 and LNCaP cells. Phytanic acid and retinoic acid synergistically inhibited the growth of both of these prostate cancer cell lines. In addition, Western blot analysis showed that both phytanic acid and DHA decreased cyclin D1 expression in PC-3 cells. We also examined retinol (vitamin A) metabolism in PC-3 cells treated with phytanic acid and DHA by HPLC. Both phytanic acid and DHA altered the metabolism of retinol. These results suggest that both phytantic acid and DHA, natural dietary RXR ligands, may be useful agents for future dietary preventive and therapeutic approaches to human prostate cancer.				
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## Introduction

Retinoids, a group of natural and synthetic small, lipophilic molecules which are vitamin A (retinol) derivatives, regulate cell proliferation and differentiation (Gudas et al., 1994). It is believed that retinoids suppress the process of carcinogenesis by inhibiting cell proliferation, enhancing apoptosis, and inducing cell differentiation (Lotan et al., 1995). Preclinical and clinical research has shown that retinoids have anticarcinogenic effects on many organ sites, including lung, breast, cervix, intestine, prostate and bladder (Kelloff et al., 1999). Moreover, epidemiological studies on human populations have demonstrated that increasing vitamin A in the diet is beneficial for the inhibition of the progression of carcinogenesis of the lung, breast, cervix, prostate, gastrointestinal tract, kidney, and oral cavity (Hong and Itri, 1994; Lotan, 1996; Niles, 2000). Retinoic acid (RA), one of the metabolites of vitamin A, exerts its physiological functions through retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Chambon, 1996). These receptors are ligand-dependent transcription factors that bind to retinoic acid response DNA elements (RAREs), thereby modulating the expression of specific downstream target genes. There are three subtypes ( $\alpha$ ,  $\beta$  and  $\gamma$ ) of each RAR and RXR, and the expression patterns of every receptor subtype are tissue specific and developmental stage specific (Dollé et al., 1990; Leid et al., 1992). These retinoid receptors function as RAR:RXR heterodimers (Mangelsdorf et al., 1994).

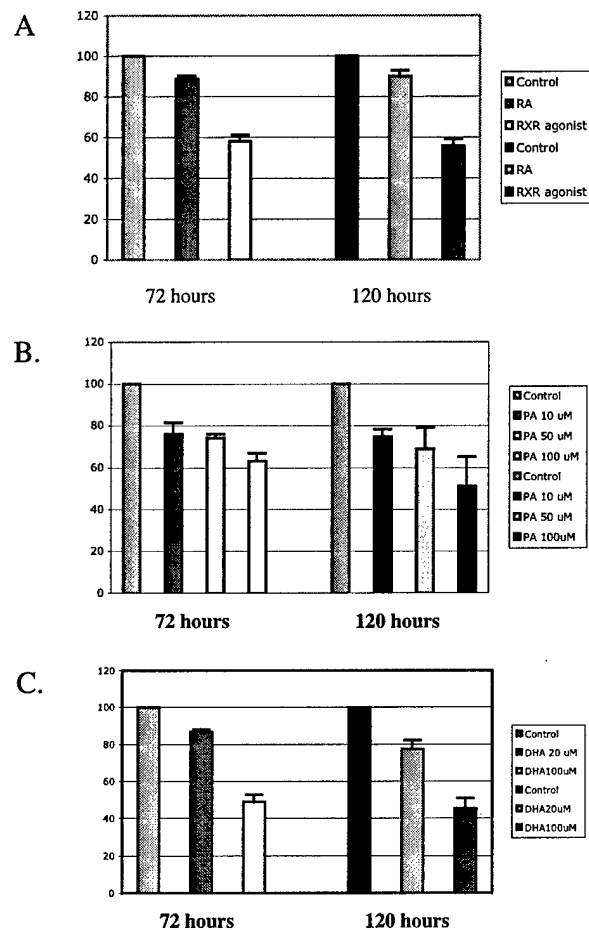
Prostate cancer is one of the most common cancers in males (Landis et al., 1998). A large body of epidemiological data indicate that retinoids have actions in the prevention of prostate cancer (Nanus and Gudas, 2000). In addition, our lab observed that the esterification of retinol to retinyl esters, and the levels of LRAT, an enzyme involved in vitamin A esterification and storage, were greatly decreased in human prostate cancer cells and tissues in sections from patient samples (Guo et al., 2002). Recent reports have shown that RXR selective retinoids represent promising agents for the prevention and treatment of prostate cancer (McCormick et al., 1999). Phytanic acid (PA) (3.7.11.15-tetramethylhexadecanoic acid) is a branched-chain, isoprenoid-derived fatty acid, generated by the oxidation of the phytol side chain of chlorophyll in mammals (Jansen et al., 1996). All phytanic acid in the human body comes from dairy products and ruminant fats in the diet (Baxter, 1968). Docosahexaenoic acid (DHA) is a long chain,  $\omega$ -3 polyunsaturated fatty acid that is present at high levels in fish oils. DHA is an essential nutrient for adequate retina and brain development (de Urquiza et al., 2000; Uauy et al., 2001). Both phytanic acid ((Kitareewan et al., 1996; Lemotte et al., 1996; Radominska-Pandya and Chen, 2002) and DHA are the RXR natural ligands (de Urquiza et al., 2000; Uauy et al., 2001).

In this project, we have observed that phytanic acid and DHA, alone and with RAR agonists, suppressed the growth of human prostate cancer cells. In addition, we found that both phytanic and DHA could inhibit the expression of cyclin D1, a cell cycle regulator. Therefore, they would be useful in the prevention and therapy of human prostate cancer. Also, the treatment of phytanic acid or DHA caused the increase of retinyl esters in prostate cancer cells and normal human prostate epithelial cells.

## Body

### 1. Cell growth analysis

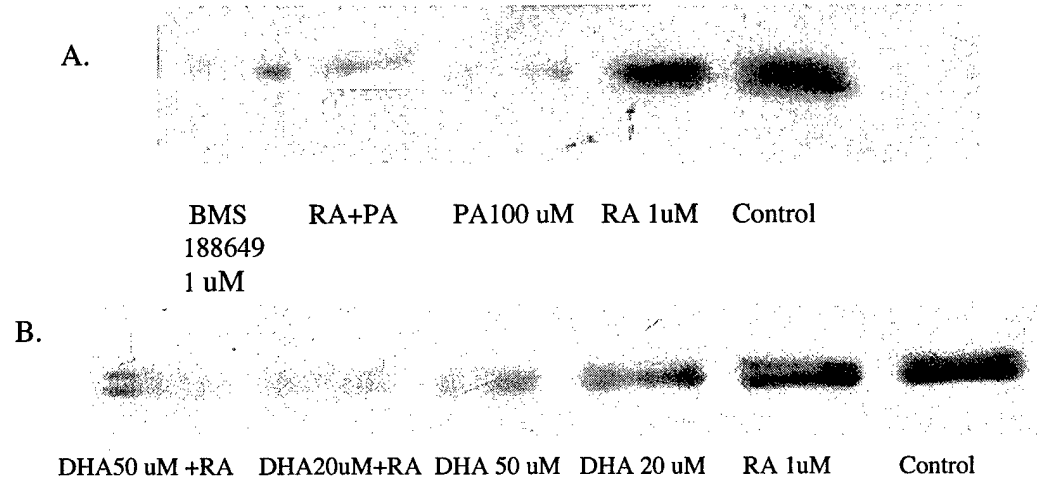
We have conducted several cell growth assays with human prostate cancer cell lines. For these experiments, we used androgen-unresponsive PC-3 cells, which in some respects simulate advanced stage prostate cancer. These cells were plated in 24 well plates in triplicate and were treated with 1  $\mu\text{M}$  *all-trans* retinoic acid (ATRA), different concentrations of phytanic acid (PA), docosahexaenoic acid (DHA), and 5  $\mu\text{M}$  of the synthetic, specific retinoid X receptor (RXR) agonist BMS 188649 (from Bristol-Myers Squibb Company) for 72 and 120 hours. Then cells were trypsinized and counted using a Coulter counter (Figure 1A). After 72 hours and 120 hours treatment, retinoic acid did not have a large effect on the growth of PC-3 cells. The RXR agonist BMS 188649 inhibited the growth of these cells at 72 and 120 hours of treatment. Seventy two and 120 hours treatment of phytanic acid or DHA also caused inhibition of the growth of PC-3 cells (Figure 1B and C) significantly.



**Figure 1. Phytanic acid, DHA, and RXR agonist BMS 188649 inhibited the growth of prostate cancer PC-3 cells.** Androgen unresponsive PC-3 cells were seeded in 24 well plates in triplicate at densities of 10,000 cells/ml/well. Then next day, cells were treated with different concentrations of phytanic acid and DHA. Fresh drugs were added every two days. After 72 and 120 hours, cells were counted using a Coulter counter.

## 2. Studies on cell cycle regulator cyclin D1

Furthermore, we investigated the mechanism of the inhibition of prostate cancer cell growth induced by phytanic acid and DHA. Cyclin D1 is a cell cycle regulator, and we tested in the presence or the absence of retinoic acid, the effects of phytanic acid or DHA on the expression level of cyclin D1 in PC-3 cells by using immunoblotting. We found that 48-hour treatment of phytanic acid (PA) or DHA alone could downregulate the expression of cyclin D1 in PC-3 cells, while retinoic acid alone did not cause significant change. However, the combination with retinoic acid increased the inhibitory effect of DHA (not phytanic acid) on the expression of cyclin D1 (Figure 2A and B). In addition, the RXR agonist BMS 188649 reduced the cyclin D1 level in PC-3 cells (Fig 2A). Taken together, the data in figures 1 and 2 demonstrated that as natural ligands of the RXRs, phytanic acid and DHA could be potential agents for the prevention and treatment of prostate cancer. Currently I am working on the effects of these two drugs on other cell cycle regulators.

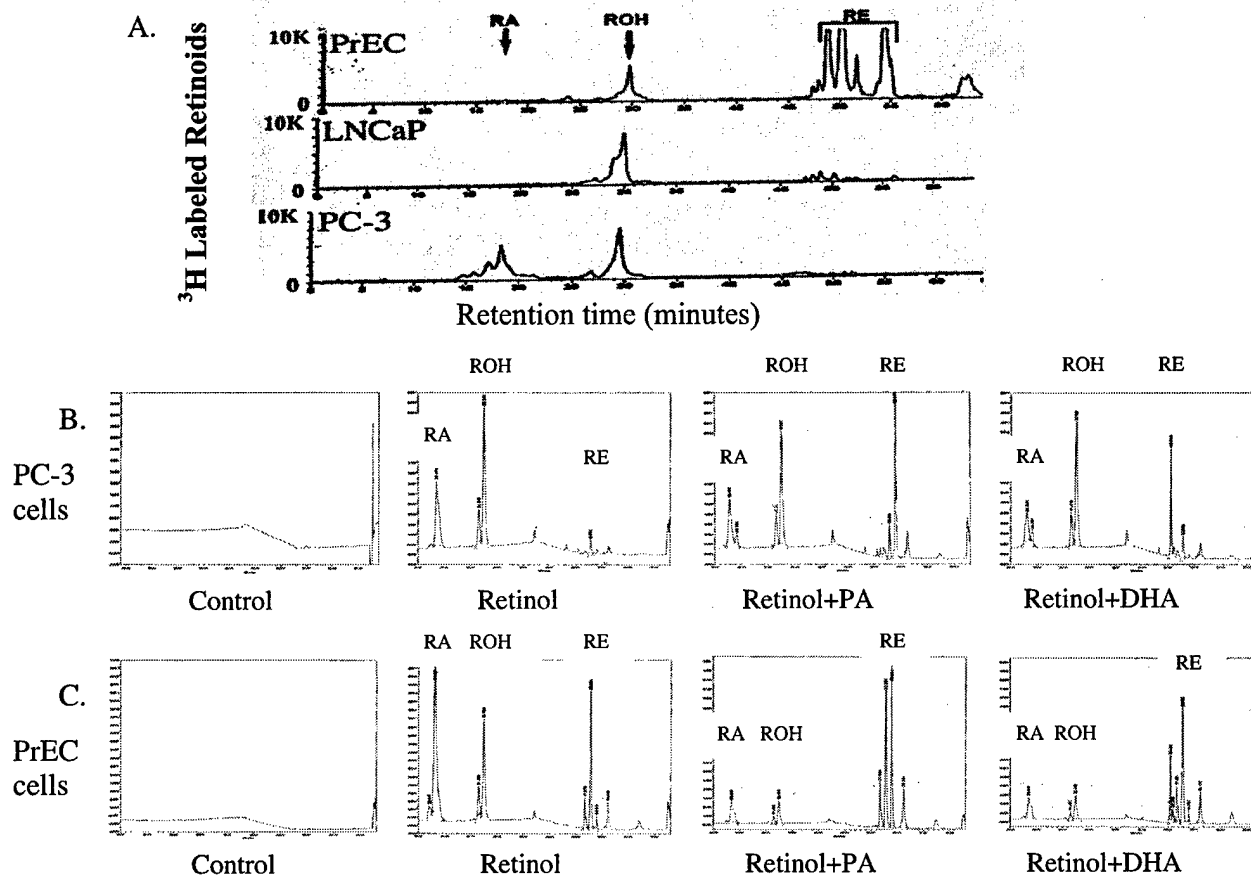


**Figure 2. Phytanic acid, DHA, and RXR agonist BMS 188649 inhibited the expression of cyclin D1 in prostate cancer PC-3 cells.** PC-3 cells were seeded in 10-cm dishes. Cells were treated with various drugs for 48 hours. Cells were harvested and the whole cell lysate protein was extracted for Western blot.

## 3. Retinol metabolism after phytanic acid and DHA treatments in normal prostate epithelial cells and prostate cancer cells

We examined retinol metabolism in cultured normal human prostate epithelial cells (PrEC), LNCaP, and PC-3 cells to determine whether differences in retinol metabolism were present by using HPLC. The normal human prostate epithelial cells (PrEC) showed the esterification of most of the retinol, and no significant production of retinoic acid was observed. Both LNCaP and PC-3 cells showed severely impaired esterification of retinol. PC-3 cells did synthesize some retinoic acid from [<sup>3</sup>H]retinol, while LNCaP cells only produced a trace amount of [<sup>3</sup>H]retinoic acid (Figure. 3A) (Guo et al., 2002). Therefore, we tested whether the treatment of phytanic acid or DHA could affect retinol metabolism in normal prostate epithelial cells (PrEC) and PC-3 cells. As

shown in figure 3 B and C, after 48 hours treatment, both phytanic acid and DHA increased the retinyl esters produced in both PrEC and PC-3 cells. Currently I am working on the mechanism of the increase of retinyl esters caused by these two drugs.



**Figure. 3** Effects of phytanic acid and DHA on the metabolism of [ $^3\text{H}$ ]retinol in normal human prostate epithelial cells (PrEC) and in human prostate cancer cells. A. Retinol metabolism in PrEC, PC-3, and LNCaP cells. B. and C., the effects of phytanic acid (PA) and DHA on retinol metabolism in PC-3 (B) and PrEC (C) cells. Cells were plated in 15 cm dishes. Cells were treated with phytanic acid (50  $\mu\text{M}$ ) and DHA (20  $\mu\text{M}$ ) in the presence of 1  $\mu\text{M}$  unlabeled retinol for 48 hours. Then cells and medium samples were harvested. The metabolism of retinol was determined by HPLC analyses of retinoids extracted from harvested cells. In B and C, all scales of Y axis are the same. RA: retinoic acid, ROH: retinol, RE: retinyl esters.

**Key research accomplishments**

We observed that phytanic acid (PA) and docosahexaenoic acid (DHA) did decrease the growth of advanced prostate cancer PC-3 cells. In addition, the RXR agonist BMS 188649 also inhibited Pc-3 cell growth.

We found that phytanic acid, DHA, as well as the RXR agonist BMS 188649, decreased the expression level of cyclin D1 in prostate cancer PC-3 cells.

We found that phytanic acid and DHA enhanced the retinyl esters produced in both PrEC and PC-3 cells.

**Reportable outcomes****Abstracts**

Tang XH and Gudas LJ (2003) Cell growth inhibition and alteration of retinoid metabolism: multiple functions of phytanic acid and docosahexaenoic acid in human prostate cancer cells. (Frontiers in Cancer Prevention Research Meeting, 2003)

Tang XH and Gudas LJ Phytanic acid and docosahexaenoic acid regulate retinol metabolism in cultured cells and mouse tissues (manuscript in preparation).

**Conclusions**

Phytanic acid and DHA suppressed the growth of human prostate cancer PC-3 cells, and downregulated the protein level of cyclin D1 in these cells. These effects are probably mediated through the activation of RXRs. In addition, phytanic acid and DHA increased the retinyl esters produced in both PrEC and PC-3 cells. These results indicate that as natural ligands of the RXRs, the dietary compounds, phytanic acid and DHA could be potential agents for the prevention and treatment of prostate cancer.

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