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## THE ROLES OF $\alpha$ -VITAMIN E AND ITS ANALOGUES IN PROSTATE CANCER

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- I. Introduction
- II. Family Members, Source, and Proper Supplemental Dose of Vitamin E
- III. General Physiological Function of Vitamin E
- IV. Vitamin E Absorption and Transport
- V.  $\alpha$ -Vitamin E-Binding Proteins
  - A.  $\alpha$ -Tocopherol Transfer Protein
  - B.  $\alpha$ -Tocopherol-Associated Protein
  - C.  $\alpha$ -Tocopherol-Binding Protein
  - D. Other Vitamin E Transport Proteins
- VI. Vitamin E and Diseases
- VII.  $\alpha$ -Vitamin E Function in Prostate Cancer: Clinical Studies
- VIII.  $\alpha$ -Vitamin E Function in Prostate Cancer: Animal Studies
- IX.  $\alpha$ -Vitamin E in Prostate Cancer: Molecular Mechanism Studies in Cancer Cells
  - A. Cellular Bioavailability of  $\alpha$ -Vitamin E and VES
  - B. Cell Cycle Arrest and DNA Synthesis Arrest
  - C. Apoptosis

*D. Signal Pathway*

*E. Invasion, Metastasis, and Angiogenesis*

X. Summary and Perspectives

References

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Prostate cancer is the second most commonly diagnosed cancer and the third leading fatal cancer in American men. Comprehensive studies from human epidemiological studies, animal tumor models, and cellular molecular levels suggested that  $\alpha$ -vitamin E and its derivatives possess remarkable chemopreventive and chemotherapeutic against prostate cancer. This chapter details the facts of  $\alpha$ -vitamin E and its nonantioxidant functions in prostate cancer, focuses on the biological mechanisms for the  $\alpha$ -vitamin E and its ester analogue,  $\alpha$ -vitamin E succinate (VES), in prevention and therapy of prostate cancer, and raises specific questions that remain for intensive investigation in the future. © 2007 Elsevier Inc.

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## I. INTRODUCTION

Vitamin E was first identified as an important nutrient for rat reproductive function in 1922 (Evans and Bishop, 1922). Although its role in human reproductive function is “not” so critical as that in rat, deficiency of vitamin E in human is associated with several human diseases, including ataxia with vitamin E deficiency (AVED), a neurodegeneration disorder (Ouahchi *et al.*, 1995). Moreover, several studies demonstrated that supplementation of  $\alpha$ -vitamin E, the major biological form of vitamin E, could reduce risks of infertility, neurological disorders, inflammation, cardiovascular diseases, diabetes, and certain types of cancers in humans (Traber and Sies, 1996; Tucker and Townsend, 2005). In 1994, the unexpected finding from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study (ATBC trial)<sup>1</sup> indicated that daily supplement of  $\alpha$ -vitamin E could reduce the incidence and mortality of prostate cancer in smoking men (The Alpha-Tocopherol,

<sup>1</sup>Abbreviations: ABCA1, ATP-binding cassette transporter A1; AR, androgen receptor; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention study; HOPE-TOO, Heart Outcomes Prevention Evaluation-The Ongoing Outcome; HPFS, Health Professionals Follow-up Study; IGFBP-3, insulin-like growth factor-binding protein-3; PLCO, Prostate, Lung, Colorectal, and Ovarian cancer-screening trial; PSA, prostate-specific antigen; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SR-BI, scavenger receptor class B type I, TAP,  $\alpha$ -tocopherol-associated protein; TBP,  $\alpha$ -tocopherol-binding protein;  $\alpha$ -vitamin E,  $\alpha$ -tocopherol TTP,  $\alpha$ -tocopherol transfer protein; VEA,  $\alpha$ -vitamin E acetate,  $\alpha$ -tocopheryl acetate; VES,  $\alpha$ -vitamin E succinate,  $\alpha$ -tocopheryl succinate; VDR, vitamin D receptor.

Beta Carotene Cancer Prevention Study Group, 1994). The following epidemiological studies, while controversial, have been supporting the protective roles of  $\alpha$ -vitamin E in prostate cancer (Chan *et al.*, 1999; Heart Protection Study Collaborative Group, 2002; Lonn *et al.*, 2005; Kirsh *et al.*, 2006). Furthermore, accumulating *in vitro* and *in vivo* evidences indicated that  $\alpha$ -vitamin E and its derivatives may function through nonantioxidant mechanisms to exhibit their antitumor roles in prostate cancer.  $\alpha$ -Vitamin E analogues, especially its ester derivative, VES, have been shown to modulate multiple signal transduction pathways to control cell growth, cell cycle, and apoptosis in prostate cancer cells. Therefore, VES may have strong chemopreventive as well as chemotherapeutic effects on prostate cancer. Here, we focus on reviewing the past and most recent studies of  $\alpha$ -vitamin E and VES in prostate cancer.

## II. FAMILY MEMBERS, SOURCE, AND PROPER SUPPLEMENTAL DOSE OF VITAMIN E

Vitamin E refers to a family of tocopherols and tocotrienols. Each sub-family is composed of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -isoforms. Vitamin E cannot be synthesized by humans and must be obtained from the diet with an abundant source found in vegetable oil, nuts, and egg yolks. The dominant form of vitamin E in the diet is  $\gamma$ -tocopherol ( $\gamma$ -vitamin E). For example, high levels of  $\gamma$ -vitamin E are found in soybean oil (10.8 mg/1 tablespoon) and corn oil (8.2 mg/1 tablespoon), while high levels of  $\alpha$ -tocopherol ( $\alpha$ -vitamin E) are found in wheat germ oil (20.3 mg/1 tablespoon), sunflower oil (5.6 mg/1 tablespoon), and almonds (7.3 mg/1 ounce) (United States Department of Agriculture (USDA) food composition database).

Although  $\gamma$ -vitamin E is the predominant isoform of vitamin E from food sources,  $\alpha$ -vitamin E is the major biological isoform in the human body. In human serum,  $\sim 90\%$  of vitamin E is  $\alpha$ -vitamin E and  $\sim 10\%$  is  $\gamma$ -vitamin E (Brigelius-Flohe and Traber, 1999). The concentration of  $\alpha$ -vitamin E in adult human serum is  $\sim 19$ – $29$   $\mu\text{M}$  (Ford and Sowell, 1999).

Vitamin E, including  $\alpha$ -vitamin E, from the natural diet is only in *RRR* configuration, whereas  $\alpha$ -vitamin E in most supplements is in synthetic form, containing eight racemic forms. The biological activity of “natural”  $\alpha$ -vitamin E is around twofold as high as “synthetic”  $\alpha$ -vitamin E. This notion is concluded from the results of classical rat fetal gestation–resorption assay, which determines the ability of different isoforms or analogues of vitamin E to maintain live fetuses in pregnant rats (Leth and Sondergaard, 1977). Vitamin E is easily oxidized and loses its antioxidant activity. To increase the stability of  $\alpha$ -vitamin E for supplements, a number of ester analogues have been synthesized, including  $\alpha$ -vitamin E acetate (VEA),  $\alpha$ -vitamin E succinate (VES), and  $\alpha$ -vitamin E nicotinate (tocopheryl nicotinate, TN).

These vitamin E analogues cannot be oxidized because the hydroxyl group, which contributes to the redox activity, has been protected with an ester bond (Prasad and Edwards-Prasad, 1992). Yet, when they go through the gastrointestinal tract, they can be converted to  $\alpha$ -vitamin E by esterase *in vivo*. To date, synthetic VEA has been used in several clinical trials, including the current Selenium and Vitamin E Cancer Prevention Trial (SELECT) for prostate cancer in the United States (Klein *et al.*, 2001; Salonen *et al.*, 2000; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994; Yusuf *et al.*, 2000).

In 2000, the US Recommended Dietary Allowances (RDA) for vitamin E was set at 15-mg  $\alpha$ -tocopherol equivalent/day for adult. On the basis of the rat fetal gestation–resorption assay, 1-IU vitamin E is defined as 1 mg (= 2.13  $\mu$ mole) of synthetic all-*rac*- $\alpha$ -tocopheryl acetate, which is equivalent to 0.45-mg (= 1.05  $\mu$ mole) *RRR*- $\alpha$ -tocopherol (Panel on Dietary Antioxidants and Related Compounds, 2000). It is reported that 200–400 IU/day of  $\alpha$ -vitamin E intake is nontoxic for humans, and this dose is commonly found in the  $\alpha$ -vitamin E supplements on the market. In the case of vitamin E deficiency or some antioxidant deficiency, one can consume  $\alpha$ -vitamin E at 800–1000 IU/day or even higher (Hathcock *et al.*, 2005). Gabsi *et al.* reported that  $\alpha$ -vitamin E supplementation (800 mg/day) in AVED patients can stabilize the neurological signs and lead to mild improvement of cerebellar ataxia, especially in early stages of the disease (Gabsi *et al.*, 2001). Unexpectedly, the results from meta-analysis of 19 clinical trials from 1966 to 2004 suggested that high doses of  $\alpha$ -vitamin E (more than 400 IU/day) will cause unexpected death (Miller *et al.*, 2005). However, this conclusion has been questioned by many researchers, particularly about the analysis methods and unhealthy participants in the clinical trials (Blatt and Pryor, 2005; DeZee *et al.*, 2005; Hemila, 2005; Krishnan *et al.*, 2005; Lim *et al.*, 2005; Marras *et al.*, 2005; Meydani *et al.*, 2005). Overall it is generally agreed that proper daily dose of vitamin E is still beneficial to individual's health.

### III. GENERAL PHYSIOLOGICAL FUNCTION OF VITAMIN E

Vitamin E in human appears to act as an antioxidant to reduce free radicals. It is the best fat-soluble antioxidant *in vivo* to intercept free radicals and prevent lipid destruction. It protects fatty acids, vitamin A, and nucleic acids from peroxidants and maintains cell membrane integrity (Dieber-Rotheneder *et al.*, 1991; Esterbauer *et al.*, 1992; Herrera and Barbas, 2001). However, vitamin E also exhibits nonantioxidant functions via modulating signaling pathways and gene expression.  $\alpha$ -Vitamin E is known to down-regulate PKC and NF- $\kappa$ B signaling (Azzi *et al.*, 2002; Chatelain *et al.*, 1993; Morante *et al.*, 2005; Tasinato *et al.*, 1995) and regulate various gene

expressions such as TTP, cytochrome P450-3A (CYP3A), CD36, scavenger receptor class B type I (SR-BI), and collagen  $\alpha 1$  (Chojkier *et al.*, 1998; Plymate *et al.*, 2003; Przyklenk and Whittaker, 2000; Ricciarelli *et al.*, 2000; Shaw and Huang, 1998; Teupser *et al.*, 1999; for review, see reference Azzi *et al.*, 2004). Several distinct biological functions of different vitamin E isoforms may be due to their unique nonantioxidant characters (Chatelain *et al.*, 1993; Tasinato *et al.*, 1995).

Among those  $\alpha$ -vitamin E ester analogues that are available on the market, VES is the most effective analogue in terms of antitumor activity (Neuzil *et al.*, 2001; Weber *et al.*, 2002; Zhang *et al.*, 2002, 2004; Zu and Ip, 2003). *In vivo* and *in vitro* studies have shown that VES inhibits growth and induces apoptosis only in carcinoma cells or transformed cells, but not in normal cells (Donapaty *et al.*, 2006; Neuzil *et al.*, 2001; Weber *et al.*, 2002; Zhang *et al.*, 2002). Further mechanistic studies indicated that VES induces apoptosis through targeting multiple molecules/signaling pathways, including transforming growth factor- $\beta$  (TGF- $\beta$ ), Fas (CD95/APO-1), the c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), and Bcl-2 family in various types of carcinoma cells (Shiau *et al.*, 2006; Yu *et al.*, 1997, 1998, 1999; Zhang *et al.*, 2002; Zu *et al.*, 2005). In addition to pro-apoptotic function, the anti-tumor activity of VES occurs through blocking cell cycle progression (Ni *et al.*, 2003; Turley *et al.*, 1997; Venkateswaran *et al.*, 2002), inducing differentiation (You *et al.*, 2001, 2002), inhibiting invasion (Zhang *et al.*, 2004), and suppressing angiogenesis (Malafa and Neitzel, 2000; Malafa *et al.*, 2002; Schindler and Mentlein, 2006) *in vitro* and/or *in vivo*. Those studies suggested that VES might be applied as a therapeutic agent for cancer treatment.

#### IV. VITAMIN E ABSORPTION AND TRANSPORT

All vitamin E isoforms from the diet have an equal uptake efficacy in the intestine, and then complex with lipoproteins to form chylomicrons, which are transported to the liver through the lymphatic system. However, in the liver, only  $\alpha$ -vitamin E is released from the hepatocytes into circulation and then supplied to the peripheral tissues. In contrast, other isoforms of vitamin E cannot be released from the liver and are metabolized and excreted into the bile and urine (Brigelius-Flohe and Traber, 1999). Therefore,  $\gamma$ -vitamin E is the most dominant form of vitamin E in the food sources, however, 90% of the vitamin E in human serum and tissues is the  $\alpha$ -vitamin E (Brigelius-Flohe and Traber, 1999). This selective effect in liver is mainly due to the presence of  $\alpha$ -tocopherol transfer protein (TTP) which has a preferentially binding ability to  $\alpha$ -vitamin E (Hosomi *et al.*, 1997; Panagabko *et al.*, 2003).

To date, intracellular transport of vitamin E has not been clearly understood. Manor group proposed that in the liver all of vitamin E isoforms are first taken up via endocytosis by SB-RI, then are transported into vesicle organelles, accumulating in lysosomes. In the lysosomes, the lipoproteins are degraded and vitamin E is exposed to TTP. TTP selectively binds and facilitates  $\alpha$ -vitamin E release from the hepatocyte via transport vesicles with the assistance of ATP-binding cassette transporter A1 (ABCA1) (Qian *et al.*, 2005). This hypothesis is based on the observation that treatment with antibodies against SR-BI could significantly reduce cellular  $\alpha$ -vitamin E amounts, and ABC transporter inhibitor, glyburide, could abrogate TTP-dependent secretion of vitamin E. In addition, they demonstrated that induction of TTP expression increases the rate of  $\alpha$ -vitamin E secretion to the media and TTP is located in the lysosomes of hepatocytes (Qian *et al.*, 2005). However, in another study, Miyazono *et al.* showed that TTP generally locates in the cytosol. This notion is concluded by the observation that TTP can translocate to lysosome/endosome after treatment with chloroquine and relocate into the cytosol after washout of chloroquine. Therefore, they proposed that  $\alpha$ -vitamin E might bind to TTP in the cytosol instead of the lysosome (Horiguchi *et al.*, 2003). Nevertheless, TTP plays a critical role in the binding, transporting, and secreting of  $\alpha$ -vitamin E in hepatocytes.

Furthermore, we found that tocopherol-associated protein (TAP) can facilitate vitamin E uptake in prostate cancer cells (Ni *et al.*, 2005). In addition, TTP, SR-BI, and ABCA1 express differentially in various prostate cancer cell lines, presumably to control the cellular bioavailability of vitamin E in prostate/prostate cancer cells as well (Ni *et al.*, 2007). Therefore, the transport of  $\alpha$ -vitamin E may utilize both vitamin E uptake genes (e.g., TAP and SR-BI) and efflux genes (e.g., TTP and ABCA1) in prostate/prostate cancer cells. The roles of those vitamin E binding proteins will be further discussed in the following section.

## V. $\alpha$ -VITAMIN E-BINDING PROTEINS

$\alpha$ -Vitamin E is a fat-soluble nutrient; however, its *in vivo* transport into cells is not only a passive process. Accumulating evidence suggested that  $\alpha$ -vitamin E transport needs the help of vitamin E-binding proteins and other lipid transfer proteins (Ouahchi *et al.*, 1995; Traber and Sies, 1996). In addition to TTP, there are several other vitamin E-binding and transport proteins, including TAP and  $\alpha$ -tocopherol-binding protein (TBP). As we found that differential expression of different vitamin E transport protein may affect cellular bioavailability of vitamin E and its consequent

antiproliferative activity, the following sections will focus on discussing the function of those vitamin E-binding/transporter proteins toward vitamin E absorption and transportation.

### A. $\alpha$ -TOCOPHEROL TRANSFER PROTEIN

TTP locates at chromosome 8q13, encoding 278 amino acids. TTP has a high expression in the liver and low expression in the brain, intestine, and other organs (Hosomi *et al.*, 1998). TTP contains a CRAL-TRIO (cellular retinal and TRIO guanine exchange factor) domain, which specifically binds small lipids. TTP has at least threefold higher binding affinity for  $\alpha$ -vitamin E than others through this CRAL-TRIO domain (Hosomi *et al.*, 1997; Panagabko *et al.*, 2003). The dissociation constant of  $\alpha$ -vitamin E to TTP is around 25 nM. Therefore, TTP can specifically bind  $\alpha$ -vitamin E and transports it out of the liver cells into circulation (Arita *et al.*, 1997). Functional loss of TTP caused by TTP point mutations in human can result in the extremely low amount of  $\alpha$ -vitamin E in serum, and eventual development of the neurological disorder, AVED (Ouahchi *et al.*, 1995; Yokota *et al.*, 2000). Consistently, TTP knockout mice have reduced  $\alpha$ -vitamin E levels in serum and peripheral tissues, and develop AVED-like syndromes (Leonard *et al.*, 2002; Yokota *et al.*, 2001).

TTP also expresses in the prostate, although not as high as in the liver. Our recent study confirmed and extended the previous reports that TTP has a more preferentially affinity for  $\alpha$ -vitamin E than  $\gamma$ -vitamin E or  $\alpha$ -vitamin E analogue, VES. This notion is supported with our finding that TTP expression levels are negatively correlated with cellular  $\alpha$ -vitamin E amount in prostate cancer cells. Overexpression or knockdown of TTP inversely regulates the cellular levels of  $\alpha$ -vitamin E, but not  $\gamma$ -vitamin E or VES, in prostate cancer cells (Ni, the Prostate in press).

### B. $\alpha$ -TOCOPHEROL-ASSOCIATED PROTEIN

TAP, also named as Sec14-like 2 (Sec14l2) or supernatant protein factor (SPF), is located at chromosome 22q12, containing 12 exons and encoding 403 amino acids. TAP has a lower expression in numerous tissues with a higher expression in the liver, brain, and prostate (Zimmer *et al.*, 2000). TAP also contains a CRAL-TRIO domain (Stocker *et al.*, 2002). However, unlike TTP, which preferentially binds  $\alpha$ -vitamin E, TAP has a broad affinity for small lipids, including  $\alpha$ -vitamin E,  $\gamma$ -vitamin E, and phosphatidylinositol (Kempna *et al.*, 2003). This notion is further supported by our observation that higher TAP expression can facilitate  $\alpha$ -,  $\gamma$ -,  $\delta$ -vitamin E and VES accumulation, and promote their antiproliferative activity in prostate cancer cells (Ni *et al.*, 2005). The dissociation constant of  $\alpha$ -vitamin E to TAP is  $\sim 0.46 \mu\text{M}$  (Zimmer *et al.*, 2000), lower than physiological  $\alpha$ -vitamin E

concentrations (19–29  $\mu\text{M}$ ) in human serum (Ford and Sowell, 1999), suggesting that TAP can bind  $\alpha$ -vitamin E and mediate vitamin E function *in vivo*. TAP is also involved in cholesterol synthesis, stimulating the activity of squalene monooxygenase and oxidosqualene cyclase (Shibata *et al.*, 2001). However, results from the *in vitro* binding assay indicated that TAP has a weak affinity for many compounds that are involved in cholesterol synthesis (Panagabko *et al.*, 2003). TAP can be cocrystallized with *RRR*- $\alpha$ -tocopheryl quinone ( $\alpha$ -TQ), the oxidation product of  $\alpha$ -vitamin E *in vivo*. Accordingly, Stocker *et al.* proposed that TAP/(SPF)'s role in cholesterol synthesis is an indirect effect: TAP/(SPF) mediates the transfer of  $\alpha$ -TQ to low-density lipoprotein (LDL), while  $\alpha$ -TQ can be oxidized to  $\alpha$ -TQH<sub>2</sub>. Thus  $\alpha$ -TQ/ $\alpha$ -TQH<sub>2</sub> protects the LDL from oxidation, resulting in reducing cholesterol uptake, eventually inducing cholesterol synthesis (Stocker, 2004; Stocker and Baumann, 2003).

Interestingly, in addition to its function to control cellular bioavailability of vitamin E, TAP can function like a tumor suppressor to control the growth of prostate cancer cells independent of its vitamin E-binding property (Ni *et al.*, 2005).

### C. $\alpha$ -TOCOPHEROL-BINDING PROTEIN

TBP, a 14.2-kDa cytosolic protein, was initially isolated from rat liver (Dutta-Roy *et al.*, 1993a). It is reported that TBP expresses in liver, heart, and human placenta (Dutta-Roy *et al.*, 1993a,b), and prefers to bind  $\alpha$ -vitamin E *in vitro*. There has been no naturally occurring TBP mutations identified in human. Overall, the function of TBP remains to be elucidated.

### D. OTHER VITAMIN E TRANSPORT PROTEINS

In addition to TTP, TAP, and TBP, many lipid transfer proteins, including SR-BI and ABCA1, also have a loose binding affinity for vitamin E.

SR-BI is the high-density lipoprotein (HDL) receptor, which mainly mediates cholesteryl ester uptake and can facilitate vitamin E uptake into the liver, brain, and intestine (Goti *et al.*, 2001; Reboul *et al.*, 2006). SR-BI knockout mice have high levels of  $\alpha$ -vitamin E in serum with significantly low vitamin E content in several organs, including ovary, testis, lung, and brain (Mardones *et al.*, 2002).

ABCA1 was reported to mediate cellular secretion of  $\alpha$ -vitamin E out of fibroblasts and macrophages (Oram *et al.*, 2001). In addition, the secretion of  $\alpha$ -vitamin E from the liver into serum is TTP- and ABCA1-dependent (Qian *et al.*, 2005). This is supported by the observation that the glyburide, the inhibitor of ATP-binding cassette transporter, completely abolished

TTP-mediated vitamin E release from hepatocytes (Qian *et al.*, 2005). Consistently, ABCA1<sup>-/-</sup> mice have undetectable levels of vitamin E in plasma (Orso *et al.*, 2000).

Our recent studies indicated that TTP, TAP, SR-BI, and ABCA1 can express in prostate cancer cells, suggesting that they may be involved in vitamin E transport in normal prostate and prostate cancer.

## VI. VITAMIN E AND DISEASES

Vitamin E is essential for maintaining numerous functions in mammals. In general, vitamin E ( $\alpha$ -vitamin E) in adult human serum is in the range of 19–29  $\mu$ M (Ford and Sowell, 1999). Low levels or deficiency of vitamin E will cause or increase the risk for various diseases, including infertility, neurological disorders, inflammation, cardiovascular diseases, diabetes, and certain types of cancers in humans (Traber and Sies, 1996; Tucker and Townsend, 2005). In humans, genetic abnormality generally underlies vitamin E deficiency. It has been reported that mutation of TTP will cause AVED, a neurodegeneration disorder (Ouahchi *et al.*, 1995). Vitamin E deficiency also happens as a consequence of fat malabsorption syndromes (Traber and Sies, 1996). For example, the patients with abetalipoproteinemia, which is due to genetic absence of apolipoprotein B (apoB) and/or apoB-containing lipidproteins, have an undetectable amount of vitamin E in their plasma (Gregg and Wetterau, 1994; Kayden, 1972).

## VII. $\alpha$ -VITAMIN E FUNCTION IN PROSTATE CANCER: CLINICAL STUDIES

To address the function of vitamin E in prostate cancer, we first address the outcome of several clinical studies (Table I). In 1994, a research group in Finland reported that daily 50-mg  $\alpha$ -vitamin E acetate supplementation decreased prostate cancer incidence by 32%, and mortality by 42% in smoking men in their large clinical trial of ATBC study (Heinonen *et al.*, 1998; The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Prostate cancer is the only cancer that can be suppressed by  $\alpha$ -vitamin E supported by this clinical trial. In the postintervention follow-up study, they found that the benefits of  $\alpha$ -vitamin E on prostate cancer prevention rapidly disappeared after discontinuation of the supplement (Virtamo *et al.*, 2003). Moreover, their results show that low levels of  $\alpha$ -vitamin E in serum increased prostate cancer mortality (Eichholzer *et al.*, 1996). In agreement with this notion, results from the  $\beta$ -carotene and retinol efficacy trial showed that the lower levels of  $\alpha$ -vitamin E in serum are significantly associated with higher incidence of prostate cancer (Goodman *et al.*, 2003). Consistently, studies

TABLE 1. Summary of Clinical Trials for the Effect of  $\alpha$ -Vitamin E Supplementation in PCa

Trial (study)	Subjects' description	Dosage and type of vitamin E	Follow-up	Risk ratio (95% CI)	Clinical outcome	References
ATBC trial, <i>n</i> = 29133	Male smokers, 50–69 years	50-mg/day <i>rac</i> - $\alpha$ -vitamin E acetate ( $\pm$ VEA)	5–8 years	0.66 (0.52–0.86)	$\alpha$ -Vitamin E supplementation reduced the incidence and mortality of PCa in smoking men. $\alpha$ -Vitamin E supplementation was associated with high level of $\alpha$ -vitamin E and $\gamma$ -vitamin E in serum and low risk of PCa. $\alpha$ -Vitamin E supplementation is associated with low level of serum hormone (androgen) in old men and a reduced risk of PCa.	Study Group (1994), Albanes <i>et al.</i> , 1995  Weinstein <i>et al.</i> , 2005  Hartman <i>et al.</i> , 2001
Postrial, <i>n</i> = 25563			6 years	0.88 (0.76–1.03)	$\alpha$ -Vitamin E-preventive role on PCa is transient, diminishes fairly rapidly following cessation of supplementation.	Virtamo <i>et al.</i> , 2003

HPFS, <i>n</i> = 47780	Healthy men, 40–75 years	0.1–15.0 IU/day	9 years	Never smokers: 0.91, current smokers/quit with past 10 years: 0.78, quit >10 year age: 2.43	$\alpha$ -Vitamin E may not prevent prostate cancer. However, $\alpha$ -vitamin E supplementation is inversely associated with the risk of metastatic or fatal prostate cancer in smoking men.	Chan <i>et al.</i> , 1999
		15.1–99.9 IU/day		Never smokers: 1.29, current smokers/quit with past 10 years: 0.51 (0.21–1.23)?, quit >10 year age: 1.33		
		$\geq 100$ IU/day		Never smokers: 1.42, current smokers/quit with past 10 years: 0.44 (0.18–1.07)?, quit >10 year age: 1.49 (metastatic/fatal cases)		
		>0–30 IU/day	8 years	Never smokers: 1.34, current smokers/quit with past 10 years: 0.67, quit >10 year age: 0.63		
PLCO trial, <i>n</i> = 29361	55–74 years	>30–400 IU/day		Never smokers: 1.16, current smokers/quit with past 10 years: 0.72, quit >10 year age: 1.03	$\alpha$ -Vitamin E supplementation did no prevent prostate cancer. However, it reduced the risk of prostate cancer in smoking men.	Kirsh <i>et al.</i> , 2006

(Continues)

TABLE 1. (Continued)

Trial (study)	Subjects' description	Dosage and type of vitamin E	Follow-up	Risk ratio (95% CI)	Clinical outcome	References
		>400 IU/day		Never smokers: 1.29, current smokers/quit with past 10 years: 0.29, quit > 10 y age: 0.95 (all advanced cases)		
Heart Protection Study, <i>n</i> = 15434	Known CDV or diabetes, 40–80 years	600 mg/day synthetic vitamin E	5 years		$\alpha$ -Vitamin E supplementation reduced 9% nonsignificant incidence of PCa and may not prevent PCa.	<a href="#">Heart Protection Study Collaborative Group, 2002</a>
HOPE trial, <i>n</i> = 9541	Known CVD or diabetes, mean age 66 years	400-IU/day <i>RRR</i> - $\alpha$ -tocopheryl acetate (+VEA)	4.5 years	0.98 (0.76–1.26)		
Extension (HOPE-TOO trial), <i>n</i> = 7030			4.5 + 2.6 years	0.90 (0.68–1.19)	Long-term supplementation of $\alpha$ -vitamin E may not prevent prostate cancer in patients with CVD or diabetes.	<a href="#">Lonn <i>et al.</i>, 2005</a>

from Switzerland on 17-year follow-up of the prospective basal study showed that the levels of  $\alpha$ -vitamin E, but not vitamin C, retinol, or carotene, were significantly low in prostate cancer patients (Eichholzer *et al.*, 1999). Furthermore, during the follow-up in the ATBC study, they also found that long-term  $\alpha$ -vitamin E supplementation decreases serum androgen (testosterone) concentrations from 573 to 539 ng/dl, suggesting that it could be one of the factors associated to the low incidence and mortality of prostate cancer in ATBC studies (Hartman *et al.*, 2001). However, whether the slightly lower concentration of androgen is a contributory factor to lower the incidence and mortality remains to be elucidated. It is worth mentioning that androgen ablation therapy did not really lower the mortality of prostate cancer.

However, recent Heart Outcomes Prevention Evaluation-The Ongoing Outcome (HOPE-TOO), Prostate, Lung, Colorectal, and Ovarian cancer-screening trial (PLCO), and Health Professionals Follow-up Study (HPFS) observed no effects of either dietary or supplemental  $\alpha$ -vitamin E on prostate cancer risk. But in the subanalyses of PLCO and HPLS trial, supplementation of  $\alpha$ -vitamin E is associated with the low incidence of prostate cancer either at advance stage or at metastatic/fatal stage in smoking men and men who recently quit smoking (Chan *et al.*, 1999; Kirsh *et al.*, 2006; Lonn *et al.*, 2005), which are consistent with the results from the ATBC trial. Taken together, those clinical trials suggested that supplement of  $\alpha$ -vitamin E might have a beneficial effect against the prostate cancer especially in smoking men. The plausible explanation is that  $\alpha$ -vitamin E may prevent prostate cancer through its antioxidant activity against oxidative stress stimulated by smoking. However, such a hypothesis cannot explain why  $\alpha$ -vitamin E has specific preventive activity against prostate cancer, but not other cancers in smoking men, suggesting that  $\alpha$ -vitamin E might regulate some specific signaling in prostate cancer.

To further elucidate whether  $\alpha$ -vitamin E and another nutrient, selenium, alone or in combination, can prevent prostate cancer, in 2001, the National Cancer Institute (NCI) began a large clinical study, SELECT. More than 32,000 men have or will participate in this trial throughout its 14 years time frame (Klein *et al.*, 2001). The trial does not use smoking status as criteria. Another large clinical trial, the Physician's Health Study II, is also ongoing, expected to be finished in 2007 (Christen *et al.*, 2000). Both trials will provide further insights into the role of  $\alpha$ -vitamin E on prostate cancer.

In the meantime, these clinical trials may have limitations to reveal the real roles of  $\alpha$ -vitamin E on prostate cancer. New clinical trials should take special consideration in (1) what is the dose, (2) which racemic form of  $\alpha$ -vitamin E (natural  $\alpha$ -vitamin E versus synthetic  $\alpha$ -vitamin E) is used, and (3) the targeted populations. More importantly, new clinical trials should go beyond the usage of  $\alpha$ -vitamin E. For example, in cultured prostate cancer cells, VES strongly suppresses cell growth, in contrast,  $\alpha$ -vitamin E or VEA, which is currently major form used in clinical trials, only slightly inhibits cancer cell growth.

New  $\alpha$ -vitamin E/VES analogues that can suppress prostate cancer cell growth *in vitro* and animal models without toxicity might be considered for inclusion in the new clinical trials in the future.

#### VIII. $\alpha$ -VITAMIN E FUNCTION IN PROSTATE CANCER: ANIMAL STUDIES

Daily oral supplementation of  $\alpha$ -vitamin E and its analogues have been applied in preclinical animal studies. For example, Nakamura *et al.* (1991) reported that a diet containing an antioxidant mix, including 0.8% catechol, 0.8% resorcinol, 0.8% hydroquinone, 2-ppm selenium, 2%  $\gamma$ -oryzanol, and 1%  $\alpha$ -vitamin E, could reduce the incidence and lesions of DMBA-initiated rat prostate carcinogenesis. Venkateswaran *et al.* showed that administration of a mix of three compounds (VES, selenium, and lycopene) in the diet dramatically inhibits prostate cancer development in Lady transgenic mice, which spontaneously develop prostate tumor (Venkateswaran *et al.*, 2004a). However, these two studies did not show whether oral intake of  $\alpha$ -vitamin E alone or VES alone has any antitumor activity *in vivo*. Another report showed that daily oral lycopene combined with  $\alpha$ -vitamin E (5 mg/kg body weight each) suppressed the orthotopic growth of PC-346C human prostate cancer in nude mice (Limpens *et al.*, 2006), but  $\alpha$ -vitamin E alone (5 or 50 mg/kg daily) did not have any impact on the tumor burden in this study. Collectively, those studies indicated that  $\alpha$ -vitamin E can be combined with other nutrients (antioxidants) to prevent prostate cancer.

Considering VES is one of the most efficient  $\alpha$ -vitamin E analogues in terms of antitumor activity *in vitro*, and has been studied *in vivo* by intraperitoneal (ip) administration in breast cancer and colon cancer studies (Weber *et al.*, 2002), we have investigated whether VES by ip administration has any effect in prostate cancer growth *in vivo*. The transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which mimics the progression of human prostate cancer and has been widely used in chemopreventive and chemotherapeutic studies (Greenberg *et al.*, 1995; Gupta *et al.*, 2001; Huss *et al.*, 2004; Mentor-Marcel *et al.*, 2001), has been applied in our study. Our results showed that ip administration of VES (50 or 100 mg/kg twice per week) could significantly inhibit the tumor burden and metastasis in TRAMP mice. This chemopreventive and therapeutic effects are accompanied by cell proliferation reduction as well as apoptosis induction. In addition, VES can increase insulin-like growth factor-binding protein-3 (IGFBP-3) levels, which are negatively associated with prostate cancer progression (Chan *et al.*, 2002); however, VES does not change IGF-1 levels in TRAMP mice. The extended investigation demonstrated that modulation of IGFBP-3 might represent one of the mechanisms for chemopreventive and chemotherapeutic activity of VES (Yin *et al.*, 2007). Importantly, VES by ip administration

of 50 and 100 mg/kg does not show chronic toxicity in mice. This conclusion is obtained from the observation that the body weight, general appearance, and histopathologic examination of liver, kidney, intestine, spinal cord, prostate, testis, and jejunum did not significantly differ between the VES-treated and vehicle control-treated mice. Consistent with the antitumor effect of VES in TRAMP mice, our results indicate that ip administration of VES (100 mg/kg twice per week) could reduce the xenograft tumor growth in nude mice (Yin *et al.*, 2007). Together, VES or analogues with similar functional structures could be a potential chemopreventive and chemotherapeutic agent for prostate cancer.

## IX. $\alpha$ -VITAMIN E IN PROSTATE CANCER: MOLECULAR MECHANISM STUDIES IN CANCER CELLS

Consistent with epidemiology and animal studies, *in vitro* cell culture system studies found that  $\alpha$ -vitamin E or its derivative, VES, could inhibit the growth of prostate cancer cells, but not normal prostate cells (Israel *et al.*, 2000; Zhang *et al.*, 2002). Several mechanisms have been proposed by different research groups to account for  $\alpha$ -vitamin E/VES antiprostata tumor activity.

### A. CELLULAR BIOAVAILABILITY OF $\alpha$ -VITAMIN E AND VES

The cellular bioavailability of drugs is an important contributor for the drug efficacy. We have examined  $\alpha$ -vitamin E and VES concentrations after treatment in different prostate cancer cells and found that cells' retention ability of  $\alpha$ -vitamin E or VES is associated with different transport gene expression and growth inhibition efficacy in prostate cancer cells. For example, prostate cancer DU145 cells are less sensitive to 20- $\mu$ M  $\alpha$ -vitamin E or VES treatment. Consistently, the cellular amount of  $\alpha$ -vitamin E and VES in DU145 cells is much lower compared to that in other prostate cancer cells after  $\alpha$ -vitamin E/VES treatment (Ni *et al.*, 2007). Therefore, the cell's uptake efficacy might be one of the factors contributing to the growth inhibitory efficacy of  $\alpha$ -vitamin E and VES.

### B. CELL CYCLE ARREST AND DNA SYNTHESIS ARREST

It was reported that VES inhibited DNA synthesis in prostate cancer LNCaP, PC-3, and DU145 cells (Israel *et al.*, 1995). In agreement with this notion, we and others showed that VES could block cell cycle progression in prostate cancer cells (Ni *et al.*, 2003; Venkateswaran *et al.*, 2002).

Venkateswaran *et al.* showed that VES could reduce the cells in S phase in both LNCaP and PC-3 cells. This S phase reduction was accompanied by G1/S phase arrest in LNCaP cells and G2/M phase arrest in PC-3 cells. Additional analysis revealed that VES significantly upregulates p27 expression, leading to the inactivation of cyclin E/cdk2, which contributes to the G1 arrest in prostate cancer cells (Venkateswaran *et al.*, 2002). Consistently, we also found that VES blocked the cell cycle at G1/S phase, and extensive examinations revealed that VES could regulate the expression of cyclin D1, D3, cdk2, cdk4, but not cdk6, resulting in disrupting Rb-E2F pathway in prostate cancer LNCaP cells. Interestingly, our results indicated that reduced cyclin D and cdk4 expression are earlier responsiveness for VES treatment (Ni *et al.*, 2003). Together, VES could alter cell cycle progression in prostate cancer cells.

### C. APOPTOSIS

In 2000, Gunawardena *et al.* showed that 3 days of racemic  $\alpha$ -vitamin E 5- $\mu$ g/ml treatment can stimulate apoptosis in actively dividing prostate cancer LNCaP cells, but not in confluent quiescent cells. They assessed nucleosome fragmentation by cell death detection ELISA as apoptosis index (Gunawardena *et al.*, 2000). In addition to  $\alpha$ -vitamin E, VES has been widely characterized as an apoptosis induction agent through different pathways involvement. In generally, the apoptosis can be induced through mitochondria-mediated, lysosomes-mediated, endoplasmic reticulum stress/cytokine signaling pathway-mediated, and extracellular death pathway-mediated manners. Zhu *et al.* proposed that VES induces prostate cancer cells apoptosis mainly through the mitochondrial pathway. This conclusion is based on the changed profiles of caspases family after VES treatment in PC-3 cells. They showed that all three executioner caspases, caspase-3, -6, -2, and the initiator caspases, caspase-8, -9, -10, but not caspase-1 or -12, were activated by VES in prostate cancer PC-3 cells (Zu and Ip, 2003). The extended study by Malafa *et al.* (2006) suggested that caspase-4 mRNA expression was also induced after VES treatment, and caspase-4 inhibitor rescued VES-induced apoptosis in PC-3 cells. Furthermore, by the microarray, Zu *et al.* identified other molecules modulated by VES. They revealed that Ask1, GADD45 $\beta$ , and Sek1, three key components of stress-activated mitogen-activated protein kinase MAPK pathway, are new targets of VES. After further analysis, they proposed that upregulated phosphorylation of Sek1 could be responsible for the JNK activation, and the activated JNK then phosphorylates its target genes, Bcl-2 and Bim, resulting in the mitochondrial translocation of Bax and Bim, consequently, inducing mitochondrial-mediated apoptosis (Zu *et al.*, 2005). In agreement with their finding, Shiao *et al.* reported that VES induced apoptosis in prostate cancer cells partly through inhibiting the function of Bcl-2 family. The mechanistic studies revealed that the interaction between Bcl-xL and Bcl-2 was blocked

by VES. Overexpression of Bcl-xL significantly rescued VES-induced apoptosis in prostate cancer LNCaP cells. Knockdown of Bcl-xL could sensitize PC-3 cells to VES-induced apoptosis. Interestingly, they used the computer model to analyze the structure of VES and Bcl-xL and found that VES could bind to Bcl-xL. Accordingly, they developed new VES derivatives with truncated side chain aiming to stabilize the binding and found that the new compounds have more proapoptosis activity compared to VES (Shiau *et al.*, 2006). Those interesting findings might need the preclinical animal model to further validate its efficacy *in vivo*.

VES could also regulate the Fas pathway in prostate cancer cells. Studies from Israel *et al.* (Israel *et al.*, 2000) showed that VES treatment triggered Fas translocation from cytosol to membrane as well as induced Fas ligand expression in prostate cancer LNCaP and PC-3 cells. In contrast, VES has no impact on normal prostate epithelial cells.

#### D. SIGNAL PATHWAY

Androgens and androgen receptor (AR) play important roles in the initiation and progression of prostate cancer. VES has been reported to inhibit the expression of AR and PSA in prostate cancer LNCaP cells. Interestingly, VES only reduced the expression of AR, but not retinol X receptor  $\alpha$  (RXR $\alpha$ ), peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ), or other steroid hormone receptors, suggesting this downregulation of AR/PSA signaling is specifically regulated by VES. Further characterizations showed that VES could regulate AR at both transcriptional and translational levels. In addition to downregulation of AR, VES upregulated VDR expression (Zhang *et al.*, 2002), suggesting that VES treatment could sensitize prostate cancer cells to low dose of vitamin D-induced antitumor activity. Currently, vitamin D has been applied for phase II clinical trial to treat prostate cancer (Trump *et al.*, 2006). The advantage of the combination treatment of VES and vitamin D is to reduce the side effects, including hypercalcemia, caused by high doses of vitamin D (Yin and Yeh, unpublished data).

The high expression levels of IGFBP-3 are inversely associated with prostate cancer progression (Chan *et al.*, 2002). Targeting on IGFBP-3 has been used as a strategy for prostate cancer therapy (Liu *et al.*, 2005). We also demonstrated that IGFBP-3 is another target gene of VES in prostate cancer cells. IGFBP-3 mRNA and protein levels can be repressed by VES treatment. Importantly, the downregulation of IGFBP-3 mRNA level is the direct VES effect since the protein inhibitor, cycloheximide, did not rescue VES-mediated reduction of IGFBP-3 mRNA levels. Strikingly, this reduction can be confirmed in preclinical animal models TRAMP and xenograft tumor mouse model (Yin *et al.*, 2007).

### E. INVASION, METASTASIS, AND ANGIOGENESIS

The primary tumor needs to degrade the extracellular matrix in order to metastasize to distal site. This step involves many matrix metalloproteinases and other proteases. The antiprostata tumor activity of VES might also come from its ability to inhibit the prostate cancer invasion and metastasis. Studies from our laboratory showed that VES could inhibit the invasion of prostate cancer cells. This effect is associated with the reduction of the activity of MMP-9, but not MMP-2, or tissue inhibitors of MMP (TIMPs) (Zhang *et al.*, 2004). Further characterization showed that VES could also inhibit cathepsins B and D activity in prostate cancer PC-3 cells (JN, unpublished data). However, how VES regulates the activities of those molecules is not well known. Angiogenesis is essential for the growth of many primary tumors and their subsequent metastases by providing oxygen and removing waste and is a target of some prostate cancer therapies. Currently, there is no report showing whether VES has antiangiogenesis effects in prostate cancer, but our *in vivo* animal studies indicate that VES might exhibit antiangiogenesis activity in prostate cancer (Yin *et al.*, 2007). Further detailed investigation of these directions will help us get insights into functional mechanisms of VES and its analogue in prostate cancer.

### X. SUMMARY AND PERSPECTIVES

In this chapter, we summarized the current knowledge of  $\alpha$ -vitamin E and its derivative, VES, in prostate cancer. VES could modulate multiple pathways to inhibit prostate cancer cell growth. However, detailed mechanisms by which VES regulates those pathways remain largely unknown. Unlike vitamins A and D, no vitamin E receptor(s) have been identified. Yet, it was reported that TAP is an  $\alpha$ -vitamin E-dependent transcriptional activator (Yamauchi *et al.*, 2001). Furthermore, our data of TAP IHC staining in clinical human prostate sample showed that TAP could locate in the nucleus (Ni *et al.*, 2005). It is possible that  $\alpha$ -vitamin E binds to TAP to turn on vitamin E-regulated genes expression. Therefore, to characterize whether TAP is a vitamin E-dependent transcriptional factor, or to identify  $\alpha$ -vitamin E receptor, if it exists, is an interesting direction. Alternatively, identification and characterization of  $\alpha$ -vitamin E-binding proteins will help obtain insights of  $\alpha$ -vitamin E's physiological roles.

It is also of great interest to systematically identify  $\alpha$ -vitamin E/VES target genes, such as via microarray methods. This could help us better understand the molecular basis of  $\alpha$ -vitamin E/VES function and to design new strategies to combine VES with other drugs. For example, several studies indicated that combination of VES and selenium may have synergistic or additive antitumor effects (Ni *et al.*, 2003; Venkateswaran *et al.*, 2004b; Zu and Ip, 2003).

Those studies suggested that combination therapy might have a benefit for treating prostate cancer, since they target on different molecules and turn on multiple pathways to inhibit prostate cancer cell growth. Currently, the SELECT trial is ongoing in United States and will be concluded in year 2013. The proposed study here may provide the molecular basis for the clinical SELECT trial.

The bioavailability of  $\alpha$ -vitamin E is important for its efficacy and biological effects. The retention ability of  $\alpha$ -vitamin E/VES is associated with its growth inhibitory efficacy in prostate cancer cells. In addition, our unpublished data indicated that  $\alpha$ -vitamin E/VES could accumulate in mouse prostate after ip administration. However, there is no report ever focusing on the detection of  $\alpha$ -vitamin E levels in human prostate tissues. It is important for us to examine whether  $\alpha$ -vitamin E could accumulate in human prostate and whether the better efficacy of  $\alpha$ -vitamin E to treat prostate cancer is associated with higher  $\alpha$ -vitamin E accumulation in human prostate, in addition to detecting serum vitamin E.

On the basis of the structural and functional analyses of VES, several VES analogues have been created (Birringer *et al.*, 2003; Shiao *et al.*, 2006; Tomic-Vatic *et al.*, 2005). Those new analogues have higher proapoptotic efficacy compared to VES *in vitro*. Although those have not yet been shown effectively in preclinical prostate cancer animal models, those new compounds will presumably lose their antitumor activity by oral consumption due to the presence of ester bond in structure as VES dose. It is thus inconvenient for them, as well as for VES, to be applied for chemopreventive purpose. Therefore, it is of great interest to develop new  $\alpha$ -vitamin E analogues that could inhibit the tumor burden without overt toxicity through oral intake. If the results are promising, those new analogues might be applied as potential chemopreventive as well as chemotherapeutic agents for prostate cancer in the future.

Recent human studies of  $\alpha$ -vitamin E yield conflicting data and suggest several factors should be taken into consideration, including target population and the usage of  $\alpha$ -vitamin E such as the dose, the form (natural form or racemic form), and stability of  $\alpha$ -vitamin E. The metabolism of  $\alpha$ -vitamin E affects the bioavailability of vitamin E and its subsequent efficacy. New  $\alpha$ -vitamin E analogues with longer half-life should be considered in the future clinical trials. On the other hand, it has been reported that  $\gamma$ -vitamin E scavenges the reactive nitrogen oxide species better than  $\alpha$ -vitamin E (Cooney *et al.*, 1993). Tocotrienols have stronger antioxidant function than  $\alpha$ -vitamin E (Suzuki *et al.*, 1993). Therefore,  $\gamma$ -vitamin E and tocotrienol may help maintain the intact structure of  $\alpha$ -vitamin E to allow it to exhibit its other functions. On another hand,  $\gamma$ -vitamin E and tocotrienols have distinct nonantioxidant functions other than  $\alpha$ -vitamin E. How to combine those different forms of vitamin E to prevent and treat prostate cancer might be another direction for future studies.

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