



### ·Original Article ·

# Reduced expression of $\alpha$ -tocopherol-associated protein is associated with tumor cell proliferation and the increased risk of prostate cancer recurrence

Xing-Qiao Wen<sup>1</sup>, Xiao-Juan Li<sup>2</sup>, Zu-Lan Su<sup>3</sup>, Yong Liu<sup>3</sup>, Xiang-Fu Zhou<sup>1</sup>, Yu-Bin Cai<sup>1</sup>, Wen-Tao Huang<sup>1</sup>, Xin Gao<sup>1</sup>

<sup>1</sup>Department of Urology, <sup>2</sup>Department of Health Care, <sup>3</sup>Department of Pathology, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, China

#### Abstract

Aim: To examine the impact and prognostic significance of  $\alpha$ -tocopherol associated protein (TAP) expression in a series of prostate cancer patients. **Methods:** Tissues from 87 patients underwent radical prostatectomy were examined for TAP expression by immunohistochemistry. The relationships of the staining results, the clinic pathological characteristics and the recurrence times were analyzed. **Results:** Compared with the adjacent areas of normal and benign glands, immunoreactivity of TAP was reduced in areas of prostate cancer. A lower TAP-positive cell number per mm<sup>2</sup> of the largest cancer area (defined as TAP-PN) was associated with higher clinical stage (r = -0.248, P = 0.0322). Inverse associations were found among the TAP-PN and positive lymph nodes (r = -0.315, P = 0.0325), preoperative prostate-specific antigen (PSA) levels (r = -0.423, P = 0.0043), tumor size (r = -0.315, P = 0.0210) and elevated tumor cell proliferation, which was indicated by the staining of Ki-67 (r = -0.308, P = 0.0026). TAP-PN was a significant predictor of recurrence univariately (P = 0.0006), as well as multivariately, adjusted for known markers including preoperative PSA, clinical stage, Gleason score, surgical margin, extra-prostatic extension, seminal vesicle invasion and lymph node metastasis (P = 0.0012). **Conclusion:** Reduced expression of TAP was associated with the cell proliferation status of prostate cancer, adverse pathological parameters and the increased risk of recurrence. (*Asian J Androl 2007 Mar; 9: 206–212*)

Keywords: α-tocopherol associated protein; prostate neoplasms; recurrence; vitamin E

#### 1 Introduction

The  $\alpha$ -tocopherol associated protein (TAP) was first identified as a tocopherol-binding protein from bovine

E-mail: xingqiaowen@yahoo.com

liver cytosol using  $\alpha$ -tocopherol as the bait [1]. Northern blotting assays indicated that higher levels of TAP mRNA were found mainly in the liver, brain and prostate. TAP might play an important physiological role in the prostate [1]. Our previous studies showed that unlike other vitamin E-associated proteins, TAP facilitated the antiproliferation effect of vitamin E and functioned like a tumor suppressor gene to control cell viability in prostate cancer [2]. Over-expression of the *TAP* gene in prostate cancer cells significantly suppressed the proliferation of the cells. Knockdown of endogenous TAP by

Correspondence to: Dr Xing-Qiao Wen, Department of Urology, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, China.

Tel: +86-20-8551-6867 ext. 2052 Fax: +86-20-8758-3378

Received 2006-07-08 Accepted 2006-08-18

small interfering RNA (siRNA) in non-malignant prostate HPr-1 cells promoted cell growth. However, little is known about the clinical significance of the expression of this novel vitamin E-binding protein in prostate cancer tissues. The aim of the present study was to examine the impact and prognostic significance of TAP expression in a series of prostate cancer patients.

Oxidative stress (OS) has been implicated in the development of many kinds of cancer, including prostate cancer [3]. Induction of high levels of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals can subject the cell to a state of OS, which might damage cellular DNA, proteins and lipids, and result in cell-cycle arrest, cell death and the development of malignancy [4]. However, what causes the increase in the susceptibility of tissues to OS is not well understood. Diet and environmental factors play important roles in the pathogenesis of prostate cancer [5]. Vitamin E is an antioxidant and had been suggested by epidemiological studies to have a protective effect against prostate cancer [6].  $\alpha$ -Tocopherol (also known as  $\alpha$ -vitamin E) is one of the compounds in vitamin E group and plays an important role in the suppression of lipid oxidation in the membranes of intracellular organelles [7]. It has been shown that vitamin E could inhibit the proliferation [8], suppress the function of androgen receptor and the expression of prostate-specific antigen [9], regulate cell cycle distribution and induce apoptosis in prostate cancer cells [10]. As a vitamin E binding protein, TAP might be involved in the procession of anti-oxidative damage. It is interesting to investigate the clinical significance of the expression of this vitamin E binding protein in prostate cancer tissues.

In the present study, we identified a novel link between a vitamin E binding protein gene *TAP*, whose expression is associated with cell proliferation, pathological parameters and the risk of recurrence in prostate cancer.

#### 2 Materials and methods

#### 2.1 Patients and tissue processing

For the present study, 87 prostate cancer specimens obtained at radical prostatectomy carried out between 2000 and 2005 were used. The patients were aged from 52 to 76 years (mean, 63.4 years). According to the International Union Against Cancer (UICC) clinical staging system (1992), the patients had cancer staged as T1a-c (n = 27), T2a-c (n = 49) and T3a (n = 11). The prostate specimens were sliced into 5-mm thick tissue blocks. The largest (dominant) focus of cancer was identified by visual inspection of the images and was chosen for subsequent immunological studies. The tissue blocks were fixed in 10% formalin and embedded in paraffin. Hematoxylin–eosin stained sections from each tissue block were evaluated for tumor histological differential status as shown by Gleason score and pathological stage (Tumor-Node-Metastasis system). Clinical recurrence was defined as a postoperative prostate-specific antigen (PSA) level > 0.4 ng/mL (Hybritech, San Diego, CA, USA) on two successive measurements with evidence of locoregional recurrences or metastases.

#### 2.2 Immunohistochemistry analysis

Immunohistochemical analysis was carried out in the largest (dominant) focus of cancer tissue in the prostate. Prostate cancer slides were used for antibodies against TAP and Ki-67 by immunohistochemistry as described previously [2]. The tissue sections were subjected to heat-induced epitope retrieval in citrate buffer (pH 6.0) using a microwave oven at 350 W for 5 min for TAP. Microwave treatment for Ki-67 was carried out for 4 min in an ethylenediaminetetraacetic acid (EDTA) buffer (pH 8.0). Endogenous peroxidase activity was blocked by incubation with 3% H<sub>2</sub>O<sub>2</sub> in methanol for 15 min, followed by a protein block (Dako Cytomation, Carpinteria, CA, USA). The primary antibodies to TAP (0.5 mg/mL, produced as described previously [2]) and Ki-67 (1 mg/mL; BioGenex, San Ramon, CA, USA) were added and incubated at room temperature for 1 h. Diaminobenzidine (DAB) and 3-amino-9-ethylcarbazole (AEC) were used as the chromogen for Ki-67 and TAP, respectively. Slides in which the primary antibody was omitted served as negative controls.

#### 2.3 Histological analysis

Each slide was evaluated independently by two pathologists in a blind manner. The mean of the results was calculated and recorded. Immunohistochemical analysis was carried out in the largest focus of cancer. For each specimen, 20–30 ocular measuring fields each composed of 100 grids and having a real area of 0.0625 mm<sup>2</sup> were randomly chosen under a microscope at a power of  $\times$  400 within a cancer. The number of TAP-positive cells per mm<sup>2</sup> of cancer tissue area was defined as TAP-PN. Ki-67 expression was estimated essentially in a similar way as

Tel: +86-21-5492-2824; Fax: +86-21-5492-2825; Shanghai, China

for TAP. The number of Ki-67-positive cells per mm<sup>2</sup> of total cancer tissue area was defined as Ki-67-PN.

#### 2.4 Statistics

Analyses were carried out using the statistical package SPSS (version 14.0, SPSS, Chicago, IL, USA). The correlation of TAP-positive cells with pathological and clinical variables was evaluated using Spearman's correlation coefficient testing. Comparisons in TAP-PN among normal and prostate cancer specimens were made using Mann–Whitney *U*-test. Univariate survival analysis of time to clinical recurrence was carried out using the product-limit method (Kaplan–Meier), with the log-rank test for differences between categories of each variable. Multivariate analyses were carried out using the Cox proportional hazard regression model. The hazard ratio and its 95% confidence interval (CI) were recorded for each marker.

#### 3 Results

#### 3.1 Staining pattern of TAP in prostate cancer tissues

Examples of immunostaining for TAP in the malignant prostate tissues are shown in Figure 1. If the immunoreactivity was positive, TAP was stained red by the chromogen of AEC in the tissues. TAP protein was mainly expressed in the prostate epithelium, and positive TAP staining was rarely seen in the stroma. In the epithelial cells, TAP staining was mostly confined to the cytoplasma compartment, not to the nuclear part. Compared with the adjacent areas of normal and benign glands, immunoreactivity of TAP was reduced in areas of prostate cancer. In areas of normal glands and benign hyperplastic glands, TAP showed strong positive cytoplasm-staining (Figure 1A, 1B). As shown in Figure 1A, 1C, prostate cancer tissues with a low Gleason grade were weakpositive in the adenocarcinoma area. In Figure 1D, normal prostate glands showed strong positive cytoplasm staining (in red). Figure 1E shows a high-grade adenocarcinoma area showing absence of TAP staining.

#### 3.2 Correlation of TAP-positive cells with clinical parameters and tumor pathology

All the 87 cases had complete pathological and clinical follow-up information and were used for analyses. This cohort consisted of 9 patients (10.3%) having positive margins, 12 (13.8%) having positive extracapsular extension, 13 (14.9%) having positive seminal vesicle



Figure 1. The  $\alpha$ -tocopherol associated protein (TAP) is lowly expressed and reversely correlated with the expression of Ki-67 in prostate cancer. (A): In the same scope, strong positive staining of TAP was seen in the benign area (TAP was stained in red by chromogen 3-amino-9-ethylcarbazole [AEC]; also shown in B), and weak-positive was shown in the adjacent area of adenocarcinoma (also shown in C). (D): Normal prostate glands were strongly positive stained in cytoplasm (in red). (E): High-grade adenocarcinoma area was absence of TAP staining. (F): Normal prostate tissue showing only a few cells were positively stained with Ki-67 in the nuclear (Ki-67 was stained in brown by chromogen diaminobenzidine. (G): Strong staining of Ki-67 in high-grade carcinoma area (in brown). (A), (D), (E): Bar = 25 µm; (B), (C), (F), (G): Bar = 10 µm.

invasion and 10 (11.5%) having positive lymph nodes. The clinical stages were distributed as 27 (31.0%) T1, 49 (56.3%) T2 and 11 (12.7%) T3a. Sixteen patients (18.4%) had a Gleason score of less than 6, 25 (28.7%) had a Gleason score of 6, 31 (35.6%) had a Gleason score of 7, and 15 (17.2%) had a Gleason score of more than 7. Preoperative PSA levels ranged from 6–180 ng/mL with a mean level of 13.5 ng/mL.

The number of TAP-positive cells per mm<sup>2</sup> of the cancer area was defined as TAP-PN. In general, patients with cancers of a relatively advanced clinical stage, a higher Gleason score, seminal vesicle invasion or lymph node involvement tended to have relatively lower TAP-PN, and some of these associations were found to be statistically significant (Table 1). The Spearman correlation analyses showed that a lower TAP-PN was associated with more advanced clinical stage (r = -0.248, P = 0.0322). Inverse associations were also found between the TAP-PN and positive lymph nodes (r = -0.231, P = 0.0325), preoperative PSA levels (r = -0.423, P = 0.0043), tumor size (r = -0.315, P = 0.0210; the tumor size refers to the volume of the entire cancer area within each specimen).

#### 3.3 Correlation of TAP-positive cells with Ki-67 antigen expression

Quantification of the proportion of cells with nuclear Ki-67 antigen expression is a measure of proliferation fraction and, hence, biological aggressiveness in malignancy [11]. Expression of Ki-67 was detected in normal prostate and prostate cancer specimens. As shown in Figure 1F, normal prostate tissue stained with Ki-67 antibody shows a few cells that were positively stained in the nucleus. In Figure 1G, much Ki-67 staining was seen in the high-grade carcinoma area.

A simple regression analysis was used to determine the relationship between the TAP and Ki-67 positive cells per mm<sup>2</sup> of cancer tissue. A scatterplot suggested that the relationship between the TAP and Ki-67 positive cell numbers could be modeled as linear. A significant reverse correlation was observed between TAP-PN and Ki-67-PN (r = -0.308, P = 0.0026).

## 3.4 TAP-PN is a predictor of clinical recurrence after surgery

The recurrence-free follow-up time for the 87 cases was 11.2-72.6 months (averaged 38.8 months). Twelve of the 87 patients (13.8%) had clinical recurrences (PSA > 0.4 ng/mL, on two successive measurements with evidence of local recurrences or metastases) during the follow-up. The value of TAP-PN as a continuous predictive marker for recurrence was analyzed separately in this set of patients using the Cox proportional hazard regression model.

Univariately, TAP-PN was inversely associated with clinical failure (hazard ratio [HR] = 0.823, HR<sup>-1</sup> = 1.215, P = 0.0006). The inversed HR of 1.215 means that for every 10 units' reduction in TAP-PN, risk of experiencing a recurrence (estimated by HR) during the follow-up time increases by 21.5%.

The TAP-PN data of this cohort were additionally

Table 1. Correlation of  $\alpha$ -tocopherol associated protein (TAP)expressing cells with clinical and pathological parameters of prostate cancer. <sup>a</sup>Spearman correlation coefficient; <sup>b</sup>Radical prostatectomy specimens.

TAP-positive cells vs.	n	ho a	P value
Clinical stage	87	-0.248	0.0322
Gleason score (cont.) <sup>b</sup>	87	-0.158	0.0922
Positive surgical margins	9	-0.027	0.8754
Extracapsular extension	12	-0.183	0.1231
Seminal vesicle invasion	13	-0.121	0.2043
Positive lymph node	10	-0.231	0.0325
Preoperative PSA (ng/mL)	87	-0.423	0.0043
Tumor volume	87	-0.315	0.0210

stratified with the median value of 64.6/mm<sup>2</sup> as a cut-off point, with 44 cases (50.6%) falling into the low category (TAP-PN  $\leq$  64.6/mm<sup>2</sup>), and 43 cases (49.4%) falling into the high (TAP-PN >  $64.6/mm^2$ ) category. A highly significant association of the low TAP-PN category with poor clinical recurrence was established by the univariate analysis  $(HR = 0.236, HR^{-1} = 4.23, P = 0.0004)$ . Thus, a patient with TAP-PN  $\leq 64.6$ /mm<sup>2</sup> had 4.2 times of more chances of experiencing a recurrence during the follow-up than the patient with a high TAP-PN level. This difference in risk can be also observed on the Kaplan-Meier plot (Figure 2). These results strongly support the hypothesis that a high number of TAP-positive cells within the cancer area was associated with a lower rate of recurrence after surgery. As expected, in this the present series of patients, other known predictors of recurrence including extracapsular extension, seminal vesicle invasion, lymph node metastasis, surgical margin status, preoperative PSA level, tumor volume and Gleason score were all significant predictors in univariate analyses.

Multivariate analysis showed that TAP-PN is a very strong independent predictor of disease progression when used either as a continuous or a grouped variable. For any two patients with identical clinical/pathological characteristics, one with low levels ( $64.6/mm^2$ ) of expression index had a 3.8 times greater chance of experiencing a recurrence during the follow-up than the other with high levels (HR = 0.265, HR<sup>-1</sup> = 3.77, *P* = 0.0012) of TAP.

Other parameters such as extra-capsular extension (HR<sup>-1</sup> = 2.252, P = 0.0201), seminal vesicle invasion (HR<sup>-1</sup> = 1.424, P = 0.0036), lymph node metastasis (HR<sup>-1</sup> = 1.932, P = 0.0431), surgical margin status (HR<sup>-1</sup> =

Tel: +86-21-5492-2824; Fax: +86-21-5492-2825; Shanghai, China



Figure 2. The  $\alpha$ -tocopherol associated protein (TAP) expression and prostate cancer progression: univariate analysis (Kaplan–Meier method) of time to clinical recurrence after radical prostatectomy in 87 patients with high or low expression level of TAP in total area stratified according to the median value of TAP-positive cell number in per mm<sup>2</sup> of the largest cancer area ( $\leq 64.6$ /mm<sup>2</sup> or > 64.6/mm<sup>2</sup>). HR: hazard ratio.

0.958, P = 0.0472), preoperative PSA level (HR<sup>-1</sup> = 1.716, P = 0.0316), tumor volume (HR<sup>-1</sup> = 2.723, P = 0.0148), and Gleason score (HR<sup>-1</sup> = 3.051, P = 0.0035), were all significant predictors in multivariate analyses.

These results indicate that TAP-PN was an independent predictor of recurrence rate after surgery. We did not analyze the survival rate during the follow-up because most of the patients were still alive when the data was collected.

#### 4 Discussion

In the present study, we found that expression of TAP at protein levels was reduced in prostate cancer tissues compared with the adjacent normal and benign tissues, which was consistent with our previous finding that both mRNA and protein levels of TAP were decreased in prostate cancer tissues [2]. According to previous studies, downregulation of the TAP gene might be specific in prostate cancer, because the mRNA levels of another two genes in the TAP family, *CRALBP* and *TTP*,

·210·

showed no significant difference between the cancers and benign tissues in seven pairs of samples [2].

We also found a significant relationship between TAP expression and the pathological parameters of prostate cancer. Reduced expression of TAP in the cancer tissues was associated with higher clinical stage, larger tumor size and elevated pre-operative PSA levels in prostate cancer patients. The number of TAP-positive cells per mm<sup>2</sup> of the cancer area was associated with elevated tumor cell proliferation as indicated by the staining of Ki-67. The patients with relatively low levels of TAP-positive cells tended to develop lymph node metastasis and were associated with the increased risk of cancer recurrence. Taken together, these results agree with the suppressive role of TAP in prostate cancer as discovered by our previous *in vitro* and *in vivo* studies [2].

We presented the possible biological reasons as to why TAP is downregulated in more advanced disease stages. Our previous study suggested that TAP suppresses PI3K/Akt signaling in prostate cancer cells, and it can control the homeostasis of phospholipid, interfere with p110a-p85 complex formation and reduce Akt activity [2]. PI3K/Akt signaling is the major survival pathway in prostate cancer cells and plays a variety of physiologic roles, such as cell growth, cell cycle regulation and survival. According to current findings, we hypothesized that no expression of TAP or insufficient function of TAP could lead to the loss of the control of PI3K/Akt signaling in the normal prostate cells, allowing the cells to transform into malignant cells, grow out of control and progress rapidly towards the end. Whether TAP is involved in other signaling pathways such as JNK or MAPK [12] remains to be investigated.

Currently, the exact physiological role of TAP protein in prostate cells is still not well-known. Yamauchi *et al.* [13] concluded that TAP has the following properties: (i)  $\alpha$ -tocopherol specific binding; (ii)  $\alpha$ -tocopherol-dependent nuclear translocation; and (iii)  $\alpha$ -tocopherol-dependent transcriptional activation in mammalian cells. It was reported that the  $\alpha$ -tocopherol metabolite,  $\alpha$ tocopherolquinone, has a higher affinity to TAP compared with  $\alpha$ -tocopherol [14]. We previously found that TAP enhanced vitamin E function in the prostate by improving vitamin E uptake [2]. As indicated in the present study, in normal prostate tissue, TAP was highly expressed in the epithelial cells, suggesting that it might facilitate the transportation of vitamin E in the prostate tissue from the plasma and retain the high concentration of vitamin E within the cells. Indeed, TAP can facilitate the retention of  $\alpha$ ,  $\gamma$ ,  $\delta$ -vitamin E, and  $\alpha$ -vitamin E succinate in the prostate cancer cells. TAP can also enhance the antiproliferation effects of  $\alpha$ -vitamin E,  $\gamma$ -vitamin E, and  $\alpha$ vitamin E succinate [2]. Wilson *et al.* [15] reported that in the rat prostate, a vitamin E deficiency disrupts some differentiation functions, such as delaying the secretion of 26 kDa protease in the ventral prostate.

Plasma and tissue vitamin E concentrations are remarkably stable in healthy humans. Three proteins, namely tocopherol regulatory proteins (TRP), have been identified and they specifically bind tocopherols. They are tocopherol transfer protein (TTP), tocopherol associated protein (TAP) and tocopherol-binding protein (TBP). They have been shown to play important roles in the tissue distribution and intracellular trafficking of vitamin E [7]. TTP mediates the selective transfer of  $\alpha$ tocopherol into plasma lipoproteins and plays an important role in the body [7]. It has been shown that a certain disease of familial isolated vitamin E deficiency (FIVE), also called ataxia with vitamin E deficiency, is caused by mutations in the gene for TTP [16]. If left untreated, these patients have extraordinarily low plasma concentrations of  $\alpha$ -tocopherol ( $\alpha$ -T), less than 1% of normal. When they take  $\alpha$ -T supplements, plasma  $\alpha$ -T concentrations reach normal levels within hours, but when they stop taking supplements, plasma  $\alpha$ -T concentrations fall to extraordinarily low levels within days. Terasawa et al. [17] reported that in TTP knockout mice, the brain was particularly susceptible to vitamin E depletion; less than 2% of the total  $\alpha$ -T in control mouse brains was detected in knockout mouse brains. Similarly as TTP, a good way to identify the role of TAP in the prostate is to establish the TAP knockout mice and carry out further studies in the animal model.

Increasing bodies of evidence have shown a prominent role for ROS in the pathogenesis of carcinoma of the prostate, which is initiated by OS [3]. However, what promotes the susceptibility of tissues to oxidative stress is not well understood. Vitamin E is an antioxidant and has been suggested in epidemiological studies to have a protective effect against prostate cancer. The incidence and mortality of prostate cancer were reduced among men receiving 50 IU of vitamin E for up to 8 years in the  $\alpha$ -tocopherol and  $\beta$ -carotene cancer prevention study (ATBC Study) [6]. These findings were unexpected and prompted the National Cancer Institute to sponsor a 12-year long-term study named Selenium and Vitamin E Cancer Prevention Trial (SELECT), in which 32 000 people were involved [18] to further identify the role of antioxidants in preventing prostate cancer.

As a vitamin E binding protein, TAP might be involved in the anti-oxidative reaction of vitamin E in the prostate. Loss of TAP expression might increase susceptibility of tissues to OS, insufficient function of TAP, which lead to deficiency in facilitating vitamin E in the body, contributing to the development and progression of prostate cancer. At present, there is little effective treatment for metastasis prostate cancer, especially for the stage of androgen-independent prostate cancer [19, 20], therefore, whether or not TAP could be developed as a valuable therapy target for prostate cancer needs to be investigated. It is interesting to elucidate how TAP exerts its effect in the reaction of anti-OS in prostate cells.

In conclusion, we have identified that reduced expression of  $\alpha$ -TAP in prostate cancer tissues is associated with adverse pathological parameters and the increased risk of cancer recurrence. TAP might be developed as a valuable marker for the prognosis of prostate cancer.

#### Acknowledgment

This study was supported by the National Natural Science Funding of China (No. 30600620) and Guangdong Natural Science Funding (No. 05001762), China.

#### References

- Zimmer S, Stocker A, Sarbolouki MN, Spycher SE, Sassoon J, Azzi A. A novel human tocopherol-associated protein: cloning, in vitro expression, and characterization. J Biol Chem 2000; 275: 25672–80.
- 2 Ni J, Wen X, Yao J, Chang HC, Yin Y, Zhang M, et al. Tocopherol-associated protein suppresses prostate cancer cell growth by inhibition of the phosphoinositide 3-kinase pathway. Cancer Res 2005; 65: 9807–16.
- 3 Miyake H, Hara I, Kamidono S, Eto H. Oxidative DNA damage in patients with prostate cancer and its response to treatment. J Urol 2004; 171: 1533–6.
- 4 Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Biol Chem 2006; 387: 365–72.
- 5 Jiang AL, Zhang PJ, Chen WW, Liu WW, Yu CX, Hu XY, *et al.* Effects of 9-cis retinoic acid on human homeobox gene *NKX3.1* expression in prostate cancer cell line LNCaP. Asian

Tel: +86-21-5492-2824; Fax: +86-21-5492-2825; Shanghai, China

J Androl 2006; 8:435-41.

- 6 Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, *et al.* Prostate cancer and supplementation with α-tocopherol and α-carotene: incidence and mortality in a controlled trial. J Natl Cancer Inst 1998; 90: 440–6.
- 7 Blatt DH, Leonard SW, Traber MG. Vitamin E kinetics and the function of tocopherol. Nutrition 2001; 17: 799–805.
- 8 Israel K, Sanders BG, Kline K. RRR-α-tocopheryl succinate inhibits the proliferation of human prostatic tumor cells with defective cell cycle/differentiation pathways. Nutr Cancer 1995; 24: 161–9.
- 9 Zhang Y, Ni J, Messing EM, Chang E, Yang C, Yeh S. Vitamin E succinate inhibits the function of androgen receptor and the expression of prostate-specific antigen in prostate cancer cells. Proc Natl Acad Sci U S A 2002; 99: 7408–13.
- 10 Malafa MP, Fokum FD, Andoh J, Neitzel LT, Bandyopadhyay S, Zhan R, *et al.* Vitamin E succinate suppresses prostate tumor growth by inducing apoptosis. Int J Cancer 2006; 118: 2441–7.
- 11 Stapleton A, Zbell P, Kattan MW, Yang G, Wheeler TM, Scardino PT, *et al.* Assessment of the biologic markers p53, Ki-67, and apoptotic index as predictive indicators of prostate carcinoma recurrence after surgery. Cancer 1998; 82:168– 75
- 12 Koochekpour S, Sartor O, Hiraiwa M, Lee TJ, Rayford W, Remmel N, et al. Saposin C stimulates growth and invasion, activates p42/44 and SAPK/JNK signaling pathways of MAPK and upregulates uPA/uPAR expression in prostate cancer and stromal cells. Asian J Androl 2005; 7: 147–58.
- 13 Yamauchi J, Iwamoto T, Kida S, Masushige S, Yamada K,

Esashi T. Tocopherol-associated protein is a ligand-dependent transcriptional activator. Biochem Biophys Res Commun 2001; 285: 295–9.

- 14 Stocker A, Baumann U. Supernatant protein factor in complex with RRR-α-tocopherylquinone: a link between oxidized vitamin E and cholesterol biosynthesis. J Mol Biol 2003; 332: 759–65.
- 15 Wilson MJ, Kaye D, Smith WE, Quach HT, Sinha AA, Vatassery GT. Effect of vitamin E deficiency on the growth and secretory function of the rat prostatic complex. Exp Mol Pathol 2003; 74: 267–75.
- 16 Copp RP, Wisniewski T, Hentati F, Larnaout A, Ben Hamida M, Kayden HJ. Localization of tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. Brain Res 1999; 822: 80–7.
- 17 Terasawa Y, Ladha Z, Leonard SW, Morrow JD, Newland D, Sanan D, *et al.* Increased atherosclerosis in hyperlipidemic mice deficient in α-tocopherol transfer protein and vitamin E. Proc Natl Acad Sci U S A 2000; 97: 13830–4.
- 18 De Francesco L. Prostate cancer prevention trial launched. Nat Med 2001; 7: 1076.
- 19 Xu XF, Zhou SW, Zhang X, Ye ZQ, Zhang JH, Ma X, *et al.* Prostate androgen-regulated gene: a novel potential target for androgen-independent prostate cancer therapy. Asian J Androl 2006; 8:455–62.
- 20 Zhao ZG, Ma QZ, Xu CX. Abrogation of heat-shock protein (HSP) 70 expression induced cell growth inhibition and apoptosis in human androgen-independent prostate cancer cell line PC-3m. Asian J Androl 2004; 6: 319–24.

Edited by Prof. Gail S. Prins