Lycopene and vitamin E interfere with autocrine/paracrine loops in the Dunning prostate cancer model[1]

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SPECIFIC AIMS

We tested lycopene and vitamin E in the Dunning prostate cancer model to prove their efficacy in prostate cancer (PCA) prevention and to identify the mechanisms by which these nutrients reduce the risk of PCA, as observed in epidemiology.

PRINCIPAL FINDINGS

30 *Copenhagen* rats were randomly assigned to five groups. The two control groups were not supplemented (control) or received placebo-supplemented diet (vehicle group). Treatment groups were fed diets enriched with 200 ppm lycopene, 540 ppm vitamin E, or both. After 4 wk of presupplementation, tumors were induced by injection of 10^5 MatLyLu prostate tumor cells into the ventral prostate lobe; supplementation was continued for an additional 18 days of tumor growth. Lycopene and vitamin E accumulation was followed by HPLC analysis. The biological activities of the nutrients in tumor tissue were evaluated by in vivo magnetic resonance (MR) imaging after 14 days of tumor growth as well as by GeneChip® analysis at trial termination 18 days after tumor induction. Gene regulations in key pathways were confirmed by TaqMan™ real-time RT-PCR.

1. Lycopene and vitamin E accumulate in tumor tissue

After 4 wk of presupplementation with vitamin E or vitamin E/lycopene cotreatment, plasma vitamin E levels increased to 47.98 and 46.44 μM, respectively. In the lycopene-supplemented groups, lycopene plasma levels of 1.02 and 0.92 μM were analyzed after the presupplementation period. Vitamin E and lycopene plasma levels both correspond to high physiological levels measured in humans.

Within 18 days of tumor growth, vitamin E and lycopene accumulated in the tumor tissue. Vitamin E levels were 75.46 and 47.60 μM in the vitamin E and the vitamin E/lycopene-treated group. Lycopene levels reached 0.38 and 0.42 μM in the two lycopene-treated groups.

2. MR imaging of tumor tissue

Fourteen days after tumor cell injection, tumors were examined in vivo by MR imaging. Vitamin E and lycopene single treatment significantly increased the necrotic area of the tumors to 36.37% and 35.97%, respectively, compared with 19.98% and 23.27% in the two control groups. The combination of vitamin E and lycopene nonsignificantly increased the necrosis rate to 27.47%.

3. GeneChip® analysis of tumor tissue

Changes in gene expression in treatment groups were analyzed relative to the expression in the vehicle group (**Table 1**). The hallmark of both the lycopene and the vitamin E effect was suppression of genes involved in steroid metabolism and signaling. Lycopene reduced steroid 5-α-reductase 1 expression in the lycopene [fold induction (fold) 0.36] and in the cotreated group (fold 0.48). Consequently, a set of androgen target genes (cystatin-related protein 1 and 2, prostatic spermine binding protein, prostatic steroid binding protein C1, C2, and C3 chain, probasin) was consistently down-regulated in both lycopene-treated groups with a fold of up to 0.02. Although changes in steroid 5-α-reductase 1 expression were insignificant in the vitamin E group, the expression of the same set of androgen target genes was down-regulated as in the lycopene-treated groups. Furthermore, vitamin E alone or combined with lycopene reduced aromatase expression (fold 0.57 and 0.65). In the cotreated group, lycopene and vitamin E acted in an additive manner on androgen target gene repression. The strong repression of

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.03-1116fje; doi: 10.1096/fj.03-1116fje

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TABLE 1. *GeneChip® analysis of MatLyLu prostate tumor tissue: changes in gene expression*

		Fold induction				
	Grouping by:	Supplementation[a]			Prostate tissue concentrations[b]	
	Reference condition:	Vehicle group			Lycopene-free prostate samples	
AFFY_ID	Description	Lyc	Vit E	Lyc/VitE	Low E	High E
J05035_g_at	Steroid 5-alpha-reductase 1 (EC 1.3.99.5)	0.88	0.67	0.48	1.04	0.50
J05035_at	Steroid 5-alpha-reductase 1 (EC 1.3.99.5)	0.41	1.03	0.64	0.39	0.33
S81448_s_at	Steroid 5-alpha-reductase 1 (EC 1.3.99.5)	0.36	1.01	0.85	1.33	0.52
M33986mRNA_at	Cytochrome p450 19 (aromatase) (EC 1.14.14.1)	0.70	0.57	0.65	0.90	0.33
rc_AI101743_s_at	Estradiol 17 β-dehydrogenase 4 (EC 1.1.1.62)	0.73	0.98	0.63	0.74	0.51
U56853_s_at	Rattus norvegicus 21-hydroxylase mRNA, complete cds	0.32	0.78	0.48	0.52	0.52
U89280_at	Oxidative 17 β hydroxysteroid dehydrogenase type 6	0.73	0.71	0.65	0.95	0.52
Z13993_f_at	Cystatin-related protein 1 precursor (CRP-1)	0.32	0.37	0.13	0.84	0.34
M58169_f_at	Cystatin-related protein 2 precursor	0.27	0.09	0.03	0.27	0.10
M58169_l_at	Cystatin-related protein 2 precursor	0.23	0.13	0.08	0.27	0.11
rc_AI639100_at	*crp2* gene for cystatin-related protein 2	0.39	0.39	0.24	0.44	0.58
J02675_at	Prostatic spermine binding protein precursor (SBP)	0.13	0.23	0.04	0.13	0.11
rc_AI639036_f_at	Best hit: rat prostatic spermine binding protein (SBP)	0.74	0.43	0.47	0.79	0.83
J00774cds_s_at	Prostatic steroid binding protein C1 chain precursor	0.07	0.05	0.02	0.07	0.13
V01255_at	Prostatic steroid binding protein C1 chain precursor	0.04	0.05	0.02	0.04	0.17
J00776cds_s_at	Prostatic steroid binding protein C2 chain precursor	0.28	0.09	0.04	0.28	0.08
X05034_at	Prostatic steroid binding protein C2 chain precursor	0.23	0.20	0.05	0.24	0.09
J00777_at	Prostatic steroid binding protein C3 chain precursor	0.47	0.18	0.09	0.25	0.19
J00772_s_at	Prostatic steroid binding protein C3 chain precursor	0.25	0.10	0.04	0.25	0.07
M27156_g_at	Probasin precursor (PB) (m-40)	0.61	0.30	0.21	0.89	0.39
M27156_at	Probasin precursor (PB) (m-40)	0.50	0.27	0.14	0.78	0.39
M26744_at	Interleukin-6 precursor (IL-6)	0.58	1.05	0.79	0.62	0.83
M15481_at	Insulin-like growth factor IA precursor (IGF-IA)	0.63	0.80	0.56	0.73	0.26
D00698_s_at	Insulin-like growth factor IA precursor (IGF-IA)	0.50	0.90	0.49	0.52	0.61
M15481_g_at	Insulin-like growth factor IA precursor (IGF-IA)	0.74	1.02	0.74	0.77	0.58
AF050159_at	Rattus norvegicus insulin receptor substrate 2 (IRS-2)	0.92	1.03	0.53	1.10	0.50
M31837_at	Insulin-like growth factor binding protein 3 precursor	1.16	1.04	1.46	1.11	1.50
rc_AI014020_f_at	Insulin 2 precursor	0.61	0.63	0.37	0.58	0.53
M25584_at	Insulin 1 precursor	0.64	0.76	1.01	0.93	1.18
E00001cds_f_at	DNA coding of rat pro-insulin	0.37	1.09	0.60	0.62	0.68

[a] Samples were grouped and analyzed according to supplementation. [b] Samples were grouped according to lycopene and vitamin E concentrations in tumor tissue (15–25 mg/L or ≥25 mg/L vitamin E) resulting in sample groups with comparable vitamin E contents, but differing in lycopene. Calculated changes in gene expression represent the effect of lycopene addition in the presence of different vitamin E concentrations.

androgen target genes was confirmed by TaqMan™ RT-PCR, as shown for prostatic spermine binding protein, prostatic steroid binding protein C2, and cystatin-related protein 2 (**Fig. 1**).

In addition to the effects on steroid metabolism and signaling, lycopene alone inhibited local IL-6 expression. Prostate-specific IGF-I expression was significantly down-regulated by lycopene alone or in combination with vitamin E.

Reduction of 5-α-reductase and IGF-I is mediated by lycopene

It has to be noted that the lycopene formulation (and, accordingly, the placebo) contained low amounts of vitamin E as stabilizer. Therefore, changes in gene expression might have been the result of a joint lyco-

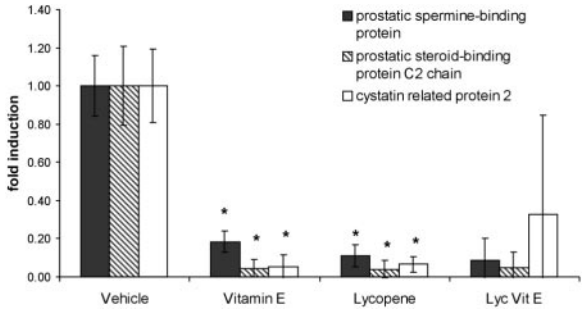

Figure 1. TaqMan™ real-time RT-PCR of selected candidate genes was used to confirm the results obtained with the GeneChip® arrays. Supplementation-induced changes of gene expression are given as fold induction relative to the expression in the vehicle-treated group ± SE (*$P<0.05$; Mann-Whitney).

pene/low vitamin E effect. To distinguish the lycopene effect from that of vitamin E, the data were regrouped in correlation to the actual vitamin E concentration in the tumors. The reorganized groups were analyzed for the lycopene activity in the presence of low (15–25 mg/L) or high (≥25 mg/L) vitamin E levels (Table 1).

This analysis confirmed the findings described above and revealed that lycopene was responsible for down-regulation of steroid 5-α-reductase 1 (fold 0.33) in prostate tumors, and thus for reduced androgen target gene expression. We confirmed that lycopene repressed IGF-I expression (fold 0.26) in tumor tissues.

CONCLUSIONS

Consistent evidence from epidemiological studies associates high intakes of vitamin E or lycopene with a reduced risk in prostate cancer. In our study of the rat Dunning prostate tumor model, lycopene and vitamin E accumulated in tumor tissue and increased necrosis of tumor tissue. We report for the first time that lycopene interfered with the prostate-specific endocrine loop of local testosterone activation and androgen signaling. Vitamin E contributed in an additive manner to the repression of androgen target genes by lycopene. This additive effect of both nutrients might be explained by their interaction at different levels of androgen signaling: whereas vitamin E, as reported, inhibits the androgen receptor, lycopene suppresses androgen activation (**Fig. 2**). Moreover, lycopene significantly reduced local expression of IGF-I and of IL-6. To confirm the epidemiologically observed risk reduction of PCA by lycopene, future clinical intervention studies should consider the local effects of lycopene rather than relying on changes in serum markers.

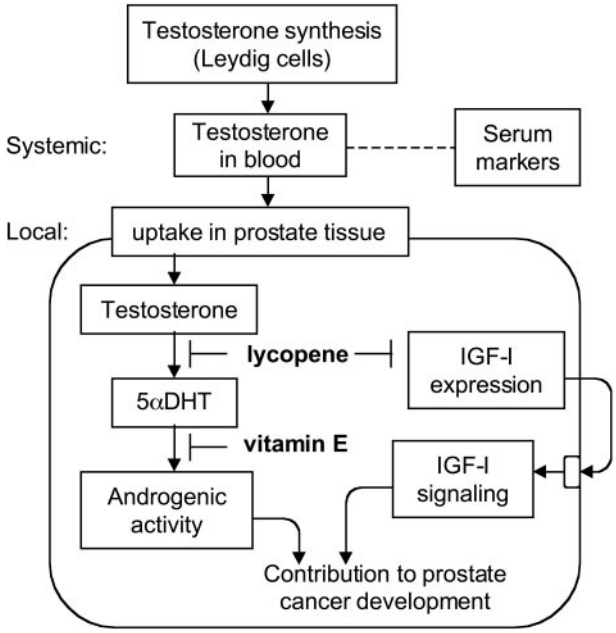

Figure 2. Schematic description of the proposed molecular mechanisms by which lycopene and vitamin E contribute to reduced prostate cancer risk. Targets inhibited by lycopene or vitamin E are indicated.

Androgen signaling is the key pathway in PCA development; growth factors IGF-I and IL-6 are discussed as risk factors for PCA development. Our findings suggest that lycopene may contribute to PCA risk reduction by interfering with prostate-specific endocrine loops of local steroid hormone and growth factor synthesis/activation and signaling. FJ