Curcumin downregulates prostate specific antigen expression through the inhibition of androgen receptor expression in human prostatic carcinoma cells

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Abstract

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Objectives

Curcumin, the active ingredient of the rhizome of the plant turmeric (Curcuma longa. Linn), exhibits anti-cancer chemopreventive effects of wide variety of tumor cells, including prostate cancer. We evaluated the effects and mechanisms of curcumin on the gene expression of PSA in human prostatic carcinoma cells.

Material & Methods

LNCaP cells were used to determine the effect of curcumin on PSA expression. Quantitative PSA expressions were assessed using enzyme linked immunosorbent assay (ELISA) and immunoblot assay. The putative gene responsive element of the deregulation of curcumin on PSA gene was identified by transient gene expression with 5'-deletion assays using a PSA reporter vector. The expression of androgen receptor (AR) and NKX3.1 was determined by RT-PCR. The effect of curcumin on the activity of AR was evaluated using electrophoretic mobility shift assay (EMSA).

Results & Conclusions

Figure 1. Curcumin blocks PSA expression with stimulation of androgen. LNCaP cells were treated with or without 1 nM R1881 and different concentrations of curcumin. (A) cells (B) media were collected for ELISA to determine the PSA levels after 24 hours treatments. (C) LNCaP cells were collected for immunoblotting assay (top) and the results were analyzed by densitometer (bottom). (D) LNCaP cells were cotransient transfected with PSA reporter vector (pPSABHE) and AR overexpression vectors. The reporter activities were determined after 20 μM of curcumin-treated (white boxes) or mock-treated (black boxes) for 24 hours.

Figure 2. Modulation of PDEF and NKX3.1 on the promoter activity of PSA gene. LNCaP cells were cotransient transfected PSA reporter vector (pPSABHE) and 0.2 μg of NKX3.1 or/and PDEF overexpression vectors. The promoter activities were determined after 24 hours without (A) or with (B) 1 nM R1881 treatments. Data are expressed as mean percent stimulation ± SE of 6 preparations of luciferase activities induced by different treatments relative to control. (** indicate p > 0.005)

Figure 3. The modulation of curcumin on the androgen response element of PSA gene in LNCaP cells. (A) PSA reporter vectors transfected-LNCaP cells were treated with mock (black bar), with 1 nM R1881 (red bar) or with R1881 and 20 μM curcumin (green bar). (B) Gene map of PSA reporter vectors. (C) PSA reporter vector (pPSABHE) transfected-LNCaP cells were treated with different concentrations of curcumin. The expression of AR were determined by immunoblot assay (top) and the results were analyzed by densitometer (bottom). (D) LNCaP cells were collected for immunoblot assay (top) and the results were analyzed by densitometer (bottom). (E) Activities of androgenic receptor after curcumin treatments were determined by EMSA assay. (** indicate p < 0.005)

Figure 4. The modulation of curcumin on the PSA gene expression is independent on the PDEF signal pathway. (A) LNCaP cells were treated with different concentration of curcumin. The expression of AR and PDEF were determined by immunoblot assay. (B) LNCaP cells were transient transfected with PDEF reporter vector (pPSABHE) and promoter activity was determined after different concentration of curcumin treatment. Data are expressed as mean percent ± SE of 6 preparations in relative to control treatment. (C) LNCaP cells were transient transfected with PDEF reporter assays and promoter activity was determined after different concentration of curcumin for 24 hour. Data are expressed as mean percent ± SE of 6 preparations in relative to control treatment. (** indicate p > 0.005)

Figure 5. Curcumin blocks the stimulation of IL-6 on the PSA gene expression. LNCaP cells were treated with 10 ng/ml IL-6 and/or 20 μM curcumin. Gene expression of PSA, AR and NKX3.1 was determined by RT-PCR (A) and immunoblot assay (B). The intracellular PSA expression was determined after 24 hours treatments of curcumin and/or IL-6 using intracellular PSA ELISA assay. (C) LNCaP cells were transient transfected with PSA reporter vector (pPSABHE). The reporter activities were determined after of curcumin and/or IL-6 treatment for 24 hours. Data are expressed as mean percent ± SE of 6 preparations in relative to control treatment. (Cur, curcumin; ** indicate p < 0.005)

Figure 6. Pathway which might involve in the regulation of curcumin on the PSA gene expression. Curcumin appear to be mediated via the androgen response element of PSA gene in LNCaP cells. Curcumin downregulates the gene expression of AR and NKX3.1 but not prostate-derived Ets factor (PDEF).