Effect of Curcumin Analog Ca27 on Androgen Receptor Translocation in Prostate Cancer Cells

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Introduction and Background

The androgen receptor (AR) plays an essential role in promoting the development and progression of metastatic prostate cancer and represents an important molecular target for therapeutic intervention. We have recently described a series of synthetic analogs of the natural product diferuloylmethane (curcumin), some of which induce the down-regulation of AR expression in prostate cancer cells by an as yet largely unknown mechanism of action. While such analogs may in the long term be lead structures for the development of therapeutic drugs, we hypothesize here that they represent ideal molecular probes to identify the mechanism(s) of action for AR down-regulation. We have previously identified the synthetic analog Ca27 as an inhibitor of the AR (see figure below), yet its mechanisms of action remain unclear. Towards this goal, we have established AR specific immunofluorescence in human prostate cancer cells and its quantitative analysis by densitometry of digitized images. These methods allowed us to test our main hypothesis whether Ca27 inhibits AR function by interfering with the translocation of the AR from the cytoplasm to the nucleus, which is part of its activation.

Methods

Cells: Human prostate cancer cells, LNCap were cultured, starved in medium without fetal bovine serum (FBS), then treated with either dimethyl sulfoxide (DMSO control), Ca27, or curcumin at 10 μM for 24 hours. Each treatment was in duplicate, such that one from each pair was induce with FBS and the synthetic androgen R1881, while the other received phosphate buffered saline (PBS) and ethanol control. Cells were then fixed with methanol.

Cytochemistry: Cells exposed to the same conditions as above were collected by trypsinization, incubated with a 0.4% trypan blue solution, and analyzed for dye exclusion capability and level of toxicity using a Countess cell-counter.

Immunofluorescence: Fixed cells were incubated in blocking buffer (Tris-buffered saline [TBS] containing 1% bovine serum albumin (BSA) and 1% Triton X-100 detergent), incubated with a polyclonal anti-AR antibody at 2ug/ml overnight at 4°C, washed in TBS with 1% Triton X-100 (TBST), incubated with an Alexa-fluor 488 conjugated secondary antibody followed by washes in TBST and brief incubation in DAPI nuclear stain to visualize the nuclei. Controls included incubation with a non-specific rabbit IgG.

Quantitative Densitometry of Digitalized Images: Images of the treated cells were captured in both the DAPI 360 excitation/460 emission filter and the GREEN 470 excitation/525 emission filter superimposed to identify nuclear regions showing AR expression.

Results

**Immunofluorescence**

Ca27/FBS/R1881 treated cells captured with the DAPI 360 excitation/460 emission filter and the GREEN 470 excitation/525 emission filter superimposed to identify nuclear regions showing AR expression.

**Quantitation of Immunofluorescence**

Signal intensity (pixel counts on the y-axis determined by densitometry using ImageJ)

**Curcumin/FBS/R1881** compared to its control: p=0.064

**Ca27/FBS/R1881** compared to its control: p=0.019

**COCLUSIONS**

- The synthetic curcumin analog Ca27 and curcumin itself significantly down-regulated the expression of AR in the nucleus at a concentration of 10μM.
- Ca27 at 10μM lead to a significant increase in cell mortality (up to 90% cell death), while curcumin at 10μM minimally affected cell viability.
- At 10μM concentrations of Ca27 and curcumin, the addition of the AR stimulators R1881 and FBS did not rescue AR down-regulation or cell death.

**FUTURE STUDIES**

- Determine the effect of Ca27 and curcumin on nuclear AR expression in dose-response analyses below the 50% inhibitory concentration (IC50).
- Determine the RATIO of nuclear to cytoplasmic AR expression for a refined analysis of AR expression vs. AR translocation.
- Complement this approach with biochemical methods, such as quantitative Western blotting for nuclear vs. cytoplasmic AR expression.