Autophagy Induction by Alpha-Santalol in Human Prostate Cancer Cells

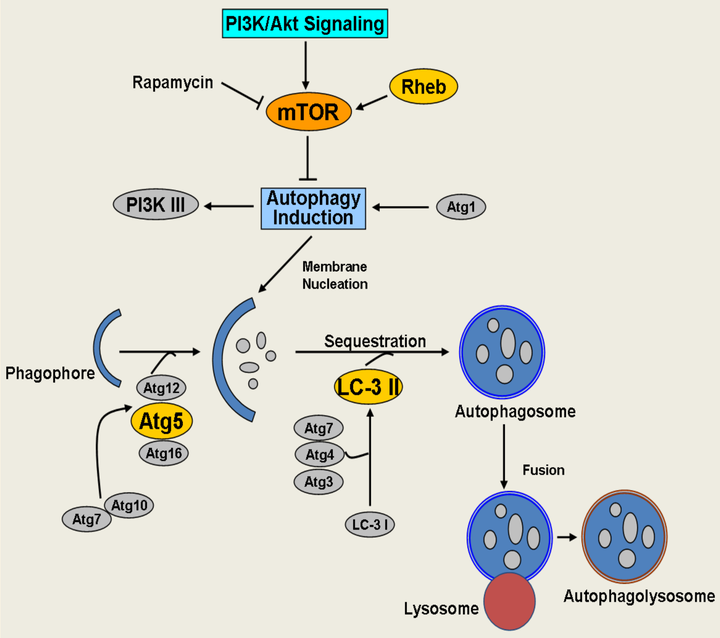
Maverick Reed, Cole Walters, Samantha Bartholomew, Sarah Hughes, and Ajay Bommareddy

Department of Pharmaceutical Sciences, Nesbitt School of Pharmacy, Wilkes University, Wilkes-Barre, PA

ABSTRACT

Alpha-santalol, a constituent of sandalwood oil, is a known inhibitor of prostate cancer in vitro, within both androgen-dependent (LNCaP) and androgen-independent (PC-3) cell lines. Our research evidence suggests alpha-santalol induces autophagy through altering the AKT-mTOR axis. Because mTOR is an inhibitor of autophagy and AKT is an activator of mTOR, as well as an inhibitor of pro-apoptotic proteins like FoxO and BAX, the reduced expression of both mTOR and AKT yields insight into alpha-santalol's currently unknown mechanism(s) of anti-tumor effects. In conclusion, our study generates support for alpha-santalol inducing autophagy in human prostate cancer cells, specifically through close association with the AKT-mTOR pathway.

PATHWAY



METHODOLOGY

Detection of acidic vesicular organelles: PC-3 and LNCaP cells($2x10^5$) were plated on cover slips in a 12 well plate and allowed to attach by overnight incubation. Following treatment with DMSO (control) or alpha- santalol for 24h, cells were stained with acridine orange in PBS, washed with PBS, and then examined under a fluorescence microscope at high power magnification for acidic vesicular organelles (i.e. autophagosomal structures).

Immunoblotting: PC-3 and LNCaP cells ($7.5x10^5$ cells for each cell line) after attachment were treated with different concentrations of α-santalol (20 and 40 µM) or DMSO for different time periods (6 or 9h). Immunoblotting was performed using standard procedures using LICOR-Odyssey infra-red scanner with actin as a loading control.

Trypan Blue Assay: PC-3 cells ($5x10^4$ cells) after attachment was pre-treated for 2 2 h with 3-MA and co-treated with alpha-santalol (20 and 40 µM) or DMSO for 24 h. Standard protocol for trypan blue assay was used for determining the cell viability in control and treatment groups.

CONCLUSIONS

- Based on our results from acridine orange staining and increased LC3 expression shown via immunoblotting, induction of autophagy was evident in both LNCaP and PC-3 cells.
- Cell viability in the presence of known autophagy inhibitor, 3MA, was significantly lower compared to alpha-santalol treated cells.
- Expression of phosphorylated (i.e. activated) AKT and mTOR proteins was reduced in cells treated with alpha-santalol comparted to control-treated cells.

This study was supported by Wilkes University's Provost Research grant and mentoring grant.

RESULTS

Figure 1: Autophagy induction in prostate cancer cells, as supported by acridine orange staining

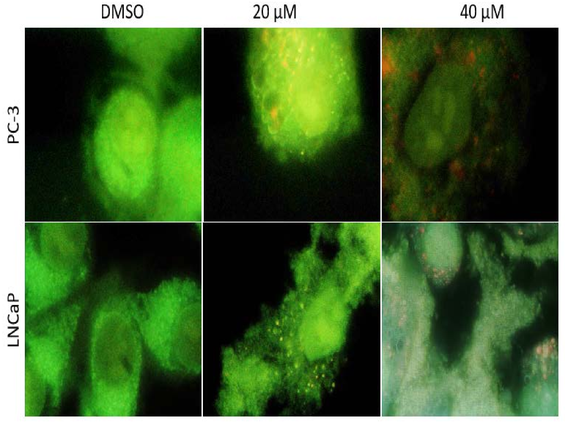


Figure 2: Effects of alpha-santalol on expression of LC3 in PC3 and LNCaP cell lines

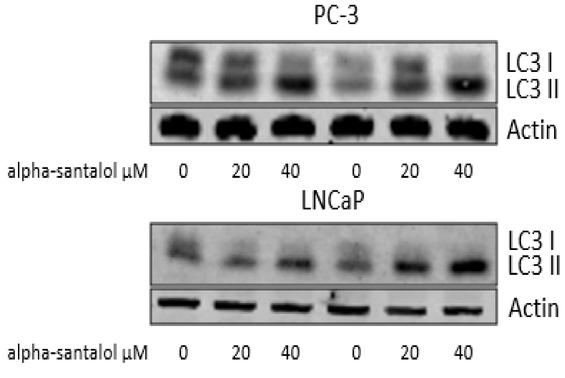


Figure 3: PC-3 cell viability in the presence of alpha-santalol and/or 3-MA

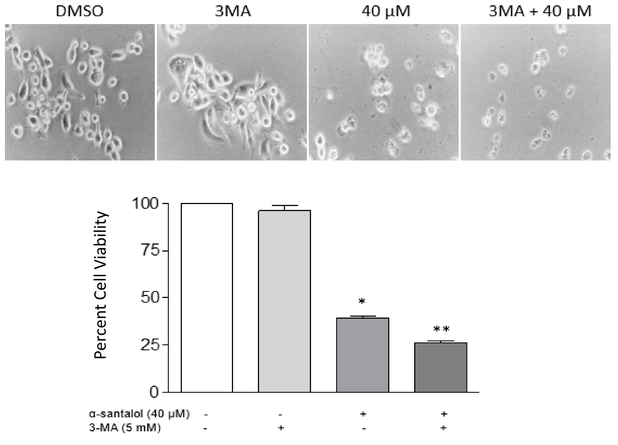


Figure 4:Effect of alpha-santalol on cellular expression of AKT-mTOR axis intermediates