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Combined N-terminal androgen receptor and autophagy inhibition increases the anti-tumor effect in enzalutamide-sensitive and enzalutamide-resistant LNCaP cells

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BACKGROUND

- Multiple androgen receptor (AR) dependent and independent resistance mechanisms limit the efficacy of castration resistant prostate cancer (CRPC) treatment.
- Novel N-terminal domain (NTD) binding AR targeting components including EPI-001 have the promising ability to block constitutively active splice variants; a major resistance mechanism in CRPC.
- Autophagy is a survival mechanism in cells exposed to anti-cancer treatment.
- We hypothesized that also a promising NTD-AR treatment may lead to up-regulation of autophagy, which can be targeted by a combination therapy with autophagy inhibitors.

MATERIALS AND METHODS

- LNCaP and LNCaP-EnzR were cultured in steroid-free medium and treated with different concentrations of EPI-001 (EPI: 10, 25, 50uM) and in combination with autophagy inhibitors chloroquine (CHQ, 20uM) or 3-methyladenine (3MA, 5mM).
- Cell proliferation was assessed by WST-1-assays after 1 and 7 days. Etidium bromide and AnnexinV were used to measure viability and apoptosis on day 7 after treatment.
- Autophagosome increase was detected by Autodot staining. In addition, autophagic activity was monitored by western blot (WB) and immunocytochemistry for the expression of LC3-I/II, Atg5 and Beclin1.

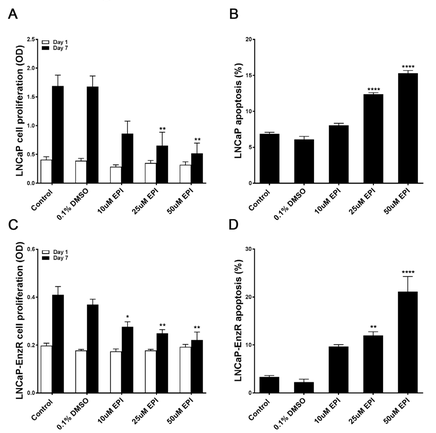

Figure 1: Cell proliferation was measured by WST-1 assay and apoptosis measured by flow cytometry using Annexin V staining. LNCaP (**A, B**) and LNCaP-EnzR (**C, D**) cells treated for up to 7 days with different concentrations of EPI. Data is shown as mean with standard error of the mean (± SEM) of three to six independent experiments. All the treatments groups were compared to untreated control at day 7. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

RESULTS

- Treatment with EPI resulted in a dose dependent reduction of cell proliferation and increase of apoptosis on day 7 in both cell lines (**Fig. 1**).
- Combination of 25uM EPI with autophagy inhibitors led to a further reduction of cell viability up to 17% for CHQ, 15% for 3MA in LNCaP and up to 24% for CHQ, 36% for 3MA in LNCaP-EnzR (**Fig. 2 A-D**).
- Assessment of autophagy levels in EPI treated cells by WB showed an increase of Atg5 and LC3-II and no change in Beclin1 expression in both cell lines (**Fig. 2 E**).
- Immunocytochemistry detected a significant increase of Atg5 and pronounced LC3-II punctuation in EPI treated LNCaP and LNCaP-EnzR (**Fig. 3**).
- This was supported by an increase in autophagosome punctuation observed by Autodot staining (**Fig 4**).

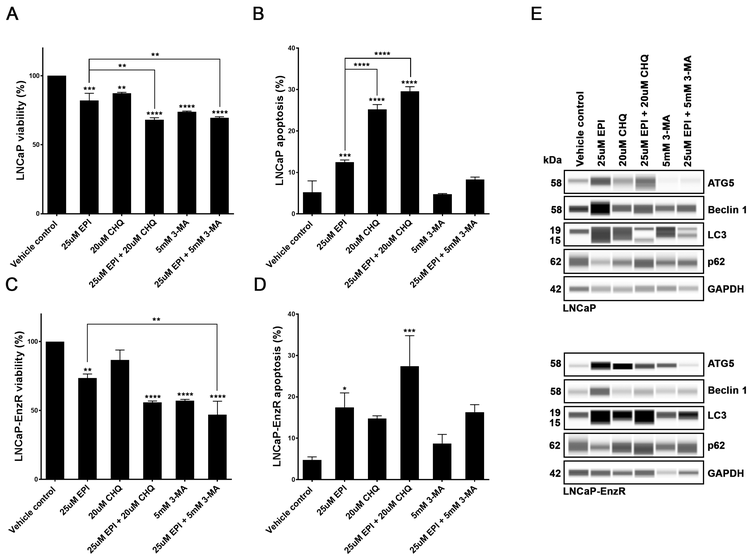

Figure 2: Viability, apoptosis and total protein expression in response to combined EPI and autophagy inhibition. Cell viability was assessed using ethidium bromide and measured by FACS (**A, C**). Apoptosis was measured by flow cytometry using Annexin V staining (**B, D**). Cells were treated with the above indicated compounds and combinations. Data is shown as mean with standard error of the mean (± SEM) of three to six independent experiments. All the treatments groups were compared to vehicle control (0.1% DMSO) at day 7. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Effects of 25µM EPI, 20µM CHQ, 5mM 3-MA and their combinations on ATG5, Beclin1, LC3 and p62 protein levels in both cell lines analyzed by immunoblotting (**E**).

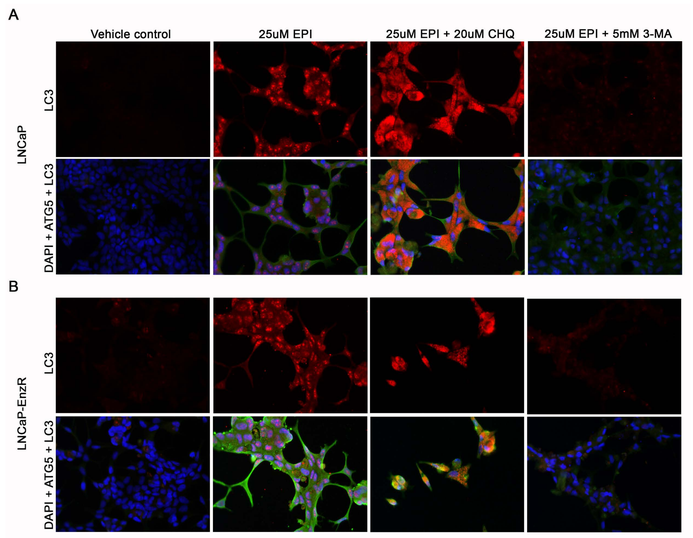

Figure 3: Visualization of LC3 and ATG5 expression using immunocytochemistry. LNCaP (**A**) and LNCaP-EnzR (**B**) cells were cultured on chamber slides and incubated for 7 days with vehicle control (0.1% DMSO), 25µM EPI, 25µM EPI + 20µM CHQ, 25µM EPI + 5mM 3-MA. Samples were stained with primary anti-ATG5 and anti-LC3B antibodies. Secondary antibodies were FITC (green) or Cy3 (red). All slides were counter-stained with DAPI (4',6-diamidino-2-phenylindole, blue).

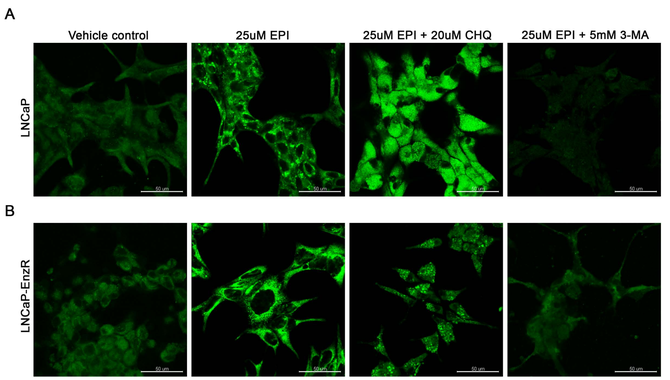

Figure 4: Visualization of autophagosome stained with AUTOdot (monodansylpentane). Confocal images of LNCaP (**A**) and LNCaP-EnzR (**B**) cells cultured on chamber slides and incubated for 7 days with vehicle control (0.1% DMSO), 25µM EPI, 25µM EPI + 20µM CHQ, 25µM EPI + 5mM 3-MA. Samples were exposed to AUTOdot staining for 15 min to visualize the formation of autophagosomes (green punctuation). Scale bars: 50 µM.

CONCLUSION

- Our data demonstrate that the treatment with EPI-001 leads to increased autophagic activity in LNCaP and LNCaP-EnzR prostate cancer cells.
- Combination of N-terminal androgen receptor blockage with simultaneous autophagy inhibition increases the antitumor effect of EPI even in LNCaP-EnzR.
- Double treatment may offer a promising strategy to overcome resistance mechanisms in advanced prostate cancer.

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