Combined N-terminal androgen receptor and autophagy inhibition increases the antitumor 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-II, Atg5 and Beclin1.

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 autophagosomal punctuation observed by Autodot staining (Fig 4).

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.