EPI-002 Accelerates Ligand Dissociation from Androgen Receptor by Disrupting N-terminus to C-terminus Interaction

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Abstract

Constitutively active splice variants of androgen receptor (AR) lacking the ligand-binding domain (LBD) are linked to the development and progression of castration-resistant prostate cancer (CRPC). Recent studies suggest a constitutively active splice variant, AR^{v567es}, is capable of interacting with full-length AR, stabilizing and enhancing its ligand-dependent activities despite castrate levels of circulating androgen. EPI-001, an AR antagonist targeting the N-terminus domain (NTD) prevents N-terminus to C-terminus (N/C) interaction of AR, which is essential for AR antiparallel dimer formation. The ligand-dependent N/C interactions slow the dissociation of ligand from the LBD. Here we examine the effect of EPI-002, the most potent stereoisomer of EPI-001, on AR^{v567es} complexed with full-length AR and test the hypothesis that EPI-002 will cause ligand to dissociate more quickly because it blocks N/C interaction. The aim of this study is two-fold as we first examined the effects of AR^{v567es} on the dissociation rate of the full-length receptor. Then, we examined the effect of EPI-002 on the ligand dissociation rate of full-length AR with and without the presence of AR^{v567es}.

We have demonstrated that EPI-002 did not affect binding affinity of wild-type full-length AR nor the time for it to reach binding equilibrium. EPI-002 accelerated the ligand dissociation rate of wild-type full-length AR possibly by disrupting N/C interaction. Co-expression of ectopic AR^{v567es} and wild-type full-length AR at 50:50 ratios did not alter the ligand dissociation rate of wild-type full-length AR but attenuated the effect of EPI-002. However, EPI-002 did not affect the ligand dissociation rate of endogenous AR in LNCaP prostate cancer cells, consistent with the lack of effect when AR has a mutation in the LBD (T877A) that enhances the N/C interaction

and slows the ligand dissociation rate compared to the wild-type AR. Together these data begin to reveal 1) the unique mechanisms of splice variant AR^{v567es} on the dissociation rate of full-length AR; and 2) the effect of an AR NTD inhibitor on the dissociation rate of full-length AR with and without the presence of splice variants.