

## HSP70/STUB1 complex regulates androgen receptor variants through proteostasis and confers enzalutamide and abiraterone resistance in lethal prostate cancer

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**Introduction & Objectives:** Proteostasis is a complementary process by which cells control the protein biosynthesis, folding, trafficking and degradation. Evidences suggested proteomic instability, such as protein misfolding and aggregation play pivotal roles in cancer cell survival and progression. AR-V7 lacking the ligand binding domain confers resistance to enzalutamide and abiraterone, and targeting AR-V7 is an ideal strategy to overcome the resistance. The mechanisms of AR-V7 proteostasis haven't been fully studied so far.

**Materials & Methods:** Expression of HSP70 and STUB1 were determined by qRT-PCR and western blot. Expression of HSP70 and STUB1 was down regulated using specific siRNA. HSP70/STUB1 and AR-V7 interaction was determined by co-immunoprecipitation and dual immunofluorescence. The gene regulating mechanisms underlying the HSP70 inhibition in drug resistant prostate cancer cells was determined by RNA sequencing analyses. The effects of HSP70 inhibition on enzalutamide sensitivity were examined in vitro and in vivo. The correlation between HSP70 and AR-V7 in high Gleason score prostate tumors was determined by qRT-PCR.

**Results:** In the present study, we analyzed enzalutamide and abiraterone resistant prostate cancer cells and found ubiquitin mediated proteolysis pathway was suppressed, and E3 ubiquitin ligases STUB1 is downregulated in enzalutamide and abiraterone resistant prostate cancer cells. STUB1 binds to AR-V7, degrades AR-V7 expression and suppresses its activity. HSP70, the STUB1 binding protein, also binds to AR-V7 and enhances AR-V7 transcriptional activity. STUB1 disassociates HSP70 from AR-V7 binding and increases AR-V7 degradation. Targeting HSP70 by siRNA or small molecular inhibitors (Apoptozole and Ver155008) significantly suppressed prostate cancer growth and improved enzalutamide and abiraterone treatment through AR-V7 inhibition in vitro and in vivo. Additionally, HSP70 is upregulated in mCRPC patients and correlates with AR-V7 level in high Gleason score and metastatic prostate tumor specimens.

**Conclusions:** Enzalutamide and abiraterone treatment induces the imbalance of AR-V7 proteostasis through the ubiquitin-proteolysis alteration. STUB1/HSP70 complex controls AR and AR variants proteostasis. Targeting HSP70 could be a valuable strategy to overcome the next generation anti-androgen resistance and improve their therapy.