

Molecular Reprogramming in Prostate Cancer Cells after Enzalutamide Exposure

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Enzalutamide, a second-generation androgen receptor (AR) antagonist, has demonstrated clinical benefit in men with prostate cancer. However, it only provides a temporary response and modest increase in survival with a rapid emergence of resistance. Studies suggest enzalutamide function as AR antagonist, but the underlying mechanisms of enzalutamide-induced molecular programming is poorly understood. Here, we show that enzalutamide stimulates expression of a novel subset of genes distinct from androgen-responsive genes. We generated a cell model of enzalutamide resistance by prolong treatment of androgen-responsive human prostate cancer LNCaP cells with progressively increasing concentration of enzalutamide (LNCaP-ENZU) and compared with parental cell line by performing Next-Gen sequencing. RNA-Seq data analysis showed that genes including XIST, AKT3, ZNF655, IRS4, HOXB3, FBN2, FHL1, GSTP1, VCAN, KIAA0408 were more than 10 fold higher (log2 fold), and 10 genes including ZNF544, KLK2, CSMD1, ZG16B, SPDEF, AR, C1R1, FOLH1, HISTIH1B, and TNPR222 were down regulated (-10 to -12 log2 fold) in LNCaP-ENZU resistant cells, compared to parental cell line. Analysis anchored with TCGA and CCLE databases, demonstrated some genes exhibited epigenetic modification/alteration in promoter methylation viz. XIST, AKT3, FOLH1 and RALYL, which were hypermethylated in prostate tumor, compared to benign prostate tissue. In context to AR, gene network analysis using 'GENEMANIA' showed the genetic interaction with AR. For example, AKT3, HOXB3, and KIAA0408 showed interaction with AR thru MTCL1 and FOLH1; whereas RALYL and KLK2 showed interaction with AR through cJUN. The differentially expressed genes of LNCaP-ENZU resistant cells overlapped with signaling pathways including IL6 signaling, glucocorticoid receptor signaling, immune response, inflammation, fatty acid signaling, drug resistance, bile acid biosynthesis, lipid metabolism, peroxisome signaling, and type II diabetes. These signaling pathways may activate downstream cytokines, transmembrane receptor and transcriptional regulators, which could further influence the expression of various target genes. Taken together, our findings demonstrate molecular reprogramming after enzalutamide exposure and identify some novel genes such as XIST, SPON2, KLK2 and ZG16B which may be used as therapeutic target to identify relapse/recurrence of castration-resistant prostate cancer after enzalutamide treatment.