

## **Genome-wide alterations in gene expression of prostate cancer (PC) cells surviving neoadjuvant androgen deprivation therapy (ADT)**

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**Background:** Although ADT initially decreases PC tumor burden, resistance and progression to castration resistant PC (CRPC) is nearly inevitable. We postulated that the stress of ADT triggers widespread alterations in expression that renders a metastable physiologic state conditioned by epigenetic changes that might be initially reversible by targeting non-androgen pathways before CRPC progression. We conducted a pilot study to explore the scope of early genome-wide expression alterations in PC foci surviving 3 months ADT (eADT).

**Methods:** mRNA from 7 frozen microdissected PC foci (>70% PC cells) and normal counterparts from men undergoing radical prostatectomy following neoadjuvant ADT were processed for RNA-seq. RNA-seq changes in eADT specimens were compared first with their normal counterparts and then with untreated PC in the TCGA PRAD (TCGA) database and to CRPC specimens in the dbGAP study phs000915.v1.p1database. The raw data (fastq files) was quantified using kallisto, normalized by TMM using R package edgeR, and batch effects corrected using R package SVA. Differential gene expression analysis was carried out using the R package sleuth. Pathway and gene set enrichment analysis by GSEA were performed using GAGE/pathview packages for Gene Ontology (GO) and KEGG.

**Results:** 5/7 eADT cases were TMPRSS2-ERG+. Prominent among the most significantly DEG in eADT compared to TCGA and mCRPC were non-coding RNAs. Among 17431 pathways, GSEAs of eADT vs. TCGA and mCRPC were, respectively, 341 (1.95%) and 1366 (7.84%) up-regulated, 46 (0.26%) and 59 (0.34%) down-regulated. Among the more well-defined KEGG pathways, eADT vs TCGA and mCRPC respectively showed 11 and 53 up-regulated and 2 and 3 down-regulated pathways. The ribosomal pathway was the only highly down-regulated pathways in eADT vs TCGA ( $q < 10^{-17}$ ), while the cell cycle and DNA replication pathways were additionally down-regulated in the eADT vs mCRPC comparison. There were 6 significantly up-regulated pathways in eADT compared to both TCGA and to mCRPC: Wnt signaling, adherence junction, steroid biosynthesis, unsaturated fatty acids, citrate cycle and ErbB signaling. Whereas eADT vs TCGA showed no other significant up-regulation, the eADT vs mCRPC showed up-regulation of calcium, MAPK, insulin, GnRH and Hedgehog signaling. Although AR full-length levels were higher than TCGA and lower than mCRPC, no significant differences in targets were apparent.

**Conclusions:** This pilot data shows that ADT triggers a wide range of gene expression alterations that support survival of PC cells with low proliferative activity and may be vulnerable to therapeutic targeting in addition to the androgen pathway. Validation of these findings is planned in a larger set of samples from the same bank.

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