RNA sequencing data of human prostate cancer cells treated with androgens

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Prostate cancer (PCa) is the most frequent cancer in North American men and PCa cells rely on the androgen receptor (AR) for growth and survival. To understand the effect of AR in cancer cells, we have treated LNCaP and LAPC4 cells, two immortalized human PCa cells in vitro, with the synthetic androgen R1881 and then performed RNA-seq analyses. High quality sequencing data have been analyzed using our bioinformatic pipeline which consists of FastQC for quality controls, Trimmomatic for trimming, and Kalisto for pseudoalignment to the transcriptome. Differentially expressed genes were identified using DESeq2 after adjustment for false-discovery rate (FDR q values < 0.05) and Relative Log Expression (RLE) normalization. Gene Set Enrichment Analysis (GSEA) was also performed to identify biological pathways significantly modulated by androgens. GSEA analyses identified the androgen signaling pathway, as well as several metabolic pathways, as significantly enriched following androgen stimulation. These analyses highlight the most significant metabolic pathways up-regulated following AR activation. Raw and processed RNA-seq data were deposited and made publicly available on the Gene Expression Omnibus (GEO; GSE128749). These data have been incorporated in a recent article describing the functions of AR as a master regulator of PCa cell metabolism. For more details about interpretation of these results, please refer to “Functional genomics studies reveal the androgen receptor as a master
regulator of cellular energy metabolism in prostate cancer” by Gonthier et al. (doi: 10.1016/j.jsbmb.2019.04.016).
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1. Data

The raw data (.fastq files) generated from Illumina sequencing were deposited on the Gene Expression Omnibus (GEO) with the reference number GSE128749 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128749). The comma separated value files (.csv) which have been produced after the quantification and pseudoalignment with the transcriptome hg38 using Kallisto were also uploaded on GEO. These files contain the raw counts, the transcripts per million (TPM) values, and the fragments per kilobase million (FPKM) values for every sample. Differentially expressed genes on normalized data were identified using a FDR q value < 0.05.

2. Experimental design, materials, and methods

2.1. Cells

LNCaP and LAPC4, two androgen receptor (AR) positive human PCa cell lines, were initially obtained from the ATCC and re-authenticated in 2016 [1]. After resuscitation, the cells were not kept in culture for more than 3 months. Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS),
streptomycin, penicillin, and sodium pyruvate in 37 °C incubators with 5% CO2. Before androgen stimulation, cells were trypsinized and seeded at a 70% confluence in RPMI-1640 media with no phenol-red and supplemental with 5% charcoal-stripped serum (CSS), streptomycin, penicillin, and sodium pyruvate, as described previously [2]. After hormonal deprivation (48h), media was changed and fresh media containing 10nM of the synthetic androgen R1881 or vehicle (EtOH 96%). 24h later, cells were harvested for RNA purification using the RNA purification kit RNeasy plus mini kit from QIAGEN.

2.2. Sequencing

Excellent RNA integrity was confirmed using a TapeStation 2200 (Agilent); all samples had an RNA integrity number equivalent (RIN®) > 8.5. mRNA enrichment and library preparation were performed using the NEBNext Ultra II Directional RNA library prep kit following the manufacturer’s protocol. RNA was then sent to the Genomic Centre of the Centre de recherche du CHU de Québec - Université Laval for sequencing using a HiSeq 2500 (125bp paired-end sequencing).

2.3. RNA-seq analysis

After sequencing, raw data were obtained in the fastq format. FastQC [3] was used for validating the quality of the data. Trimming of the adaptor content and over-represented sequences was performed using Trimmomatic [4]. Also note that trimming was performed with the minimal length (MINLEN) set at 36. Quality check using FastQC was performed again on the trimmed sequences (Table 1). For the pseudoalignment of the trimmed sequences to the hg38 transcriptome, the Kallisto tool was used [5]. Final normalization was performed using the Relative Log Expression (RLE) method [6]. We have used the R-package called Tximport to convert the transcript quantifications to gene quantifications [7].

2.4. Differential gene expression and GSEA analysis

To study genes regulated by AR in PCa cells, differential expressed genes were identified using a FDR q value < 0.05 with DESeq2 [8]. Overall, 1868 and 716 genes were up-regulated in LNCaP and LAPC4 cells and 2294 and 847 genes were significantly down-regulated in LNCaP and LAPC4 cells, respectively (Fig. 1A). Of these, 321 common genes were up-regulated while 314 common genes were down-regulated in both cell lines (Fig. 1B). GSEA analyses [9] were also performed using TPM values to identify the most significantly up-regulated pathways following activation of AR in these human PCa cells. In both cell lines, the androgen signaling pathway was highly enriched following R1881 treatment (Fig. 1C). In addition, several metabolic pathways were also enriched in both LNCaP and LAPC4 cells following AR activation (Fig. 1D).

| Table 1 |
| Number of reads for raw and trimmed sequences of PCa cells treated with androgens. |

| Cell lines | Treatment   | Reads (Raw) | Reads (after trimming) |
|------------|-------------|-------------|------------------------|
| LNCaP      | Control #1  | 9327182     | 7694732                |
| LNCaP      | Control #2  | 11058265    | 9014786                |
| LNCaP      | Control #3  | 10258931    | 8909738                |
| LNCaP      | R1881 #1    | 7812714     | 6616969                |
| LNCaP      | R1881 #2    | 9964804     | 8508748                |
| LNCaP      | R1881 #3    | 10496859    | 8965255                |
| LAPC4      | Control #1  | 8744802     | 7390781                |
| LAPC4      | Control #2  | 6528343     | 5443146                |
| LAPC4      | Control #3  | 10146342    | 8641030                |
| LAPC4      | R1881 #1    | 9384625     | 7940193                |
| LAPC4      | R1881 #2    | 11134285    | 9474978                |
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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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