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Data Article

Bulk mRNA-seq data from wild-type and prostate cancer-developing mice reveal a reprogramming of the estrogen and androgen responses after carcinogenesis

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ABSTRACT

Sex hormones are necessary for the development and functions of the normal prostate as well as for the initiation and progression of prostate tumors. Indeed, androgens and estrogens can activate their respective nuclear receptors to modulate the expression of multiple genes and pathways in prostate cells. Nevertheless, the androgen and estrogen responses in the normal prostate, and the transcriptomic changes occurring after carcinogenesis, remain poorly understood. Here, wildtype mice and transgenic mice that spontaneously develop prostate cancer (C57BL/6J PB-Cre4$^{+/-}$;*Pten*$^{fl/fl}$) were castrated to ensure hormone deprivation. After three days, animals received injections of testosterone and/or estradiol. After one day, the prostates were harvested, and RNA was purified for sequencing. Sequencing data were then analyzed to study transcriptional modulations following hormonal exposures in normal and tumoral murine prostates. New analyses can be carried out with specific fold-change thresholds for gene expression, or with different pair-wise combinations between conditions (treatments and/or mouse models). Together, the data

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generated herein are a useful tool to study hormonal transcriptional responses in prostate and prostate cancer biology.

Specifications Table

Subject	Cancer Research.
Specific subject area	Hormonal transcriptomic responses (androgenic and estrogenic) in murine prostate, and changes in a tumoral context.
Type of data	Bulk mRNA-seq, Figures Raw and analyzed
Data collection	Normal and prostate cancer-developing mice were castrated to ensure steroid depletion. Three days after, mice were injected with hormones, and, following 24 h, they were sacrificed and their prostates harvested. The tissues were then used for RNA purification, before sequencing with a NovaSeq 6000. Verification of mRNA-seq data quality and cleaning were realized with FastQC, MultiQC,and Trim_Galore/Cutadapt. Kallisto was used for pseudo-alignment of reads with the Gencode vM25 murine reference transcriptome. Gene Set Enrichment Analysis tool was employed to study transcriptomic changes, using normalized counts.
Data source location	• Institution: Next Generation Sequencing Platform of the CRCHUQ-Université Laval • City/Town/Region: Québec City, QC • Country: Canada
Data accessibility	Repository name: Gene Expression Omnibus Data identification number: GSE254635 Direct URL to data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE254635
Related research article	C. Lafront, L. Germain, G.H. Campolina-Silva, C. Weidmann, L. Berthiaume, H. Hovington, H. Brisson, C. Jobin, L. Frégeau-Proulx, R. Cotau, K. Gonthier, A. Lacouture, P. Caron, C. Ménard, C. Atallah, J. Riopel, É. Latulippe, A. Bergeron, P. Toren, C. Guillemette, M. Pelletier, Y. Fradet, C. Belleannée, F. Pouliot, L. Lacombe, É. Lévesque, É. Audet-Walsh, The estrogen signaling pathway reprograms prostate cancer cell metabolism and supports proliferation and disease progression, J Clin Invest. 134(11):e170809.

1. Value of the Data

- The androgen receptor (AR) is central to prostate and prostate cancer biology, including resistance to AR-targeted therapies. Since AR is a transcription factor, the dataset presented herein allows a better understanding of the functional role of AR in prostate physiology and disease.
- It appears clear now that estrogens, as androgens, are essential for prostate and prostate cancer biology. However, little is known about the estrogen-induced transcriptomic response in these two contexts. Sequencing data presented herein can help better understand the molecular and cellular functions of estrogens in both settings.
- Research projects regarding the impact of transcriptomic changes in sex hormone-dependent tissues or cancers, such as in the prostate, can benefit from these data.
- It is possible to choose specific pair-wise comparisons between treatments and/or mouse models, as well as select a precise fold-change threshold to generate significant gene expression lists to study the different sex hormone's impact on the normal and tumoral prostate transcriptome.

2. Background

Androgens and estrogens are both important in the normal prostate, but the transcriptomic responses induced by these two hormones are still poorly understood. Furthermore, little is known about the transcriptomic changes of the androgen and estrogen responses following carcinogenesis. Indeed, prostate cancer (PCa) is known as a hormone-dependent disease, since it requires the action of both the androgen and estrogen receptors (AR and ERα), two transcription factors that can modulate the expression of multiple genes and biological pathways once activated [1–3]. To better understand the sex hormone-induced transcriptional responses in the normal and tumoral prostate, bulk mRNA-sequencing data of AR+/ERα+ prostates from wild-type (WT) and PCa-developing mouse models were generated. To this end, mice were castrated before receiving injections of androgens and/or estrogens. After one day, mice were sacrificed and their prostates were harvested. RNA was then purified and sequenced. The dataset presented herein allows a better appreciation of the androgen and estrogen responses in the normal murine prostate, as well as the reprogramming of these hormonal responses in a tumoral context.

3. Data Description

The raw data used for this manuscript are available on the Gene Expression Omnibus (GEO) repository and can be accessed with the reference number GSE254635 [4]. It consists of 30 samples. Sixteen are from normal mouse prostates which received four different treatments (vehicle, estradiol, testosterone and estradiol + testosterone). There are four replicates per treatment, each replicate representing one prostate sample. The remaining 14 samples are from tumoral mouse prostates. There are three replicates for vehicle and testosterone and four replicates for estradiol and estradiol + testosterone. For each sample, raw fastq files are available, as well as normalized and raw quantified counts in CSV format. The related research article associated with this dataset presented the upregulated pathways of the androgen and estrogen responses in both normal and tumoral murine prostates [1]; herein, we focused on the downregulated pathways induced by these two hormones in both settings (Fig. 1 for normal prostate and Fig. 2 for tumoral prostate), using the Gene Set Enrichment Analysis (GSEA) software [5].

4. Experimental Design, Materials and Methods

4.1. Mouse models and animal housing

Two mouse models were used for this study: C57BL/6J *Pten*$^{fl/fl}$ (WT) and C57BL/6J PB-Cre4$^{+/-}$;*Pten*$^{fl/fl}$ (developing PCa, generated by Wang and colleagues [6]). The latter model undergoes a deletion of the tumor suppressor gene *Pten* exclusively in prostate luminal epithelial cells. This loss of *Pten* leads to the development of a localized adenocarcinoma after about 24 weeks of age. This particular *in vivo* model is a good depiction of the evolution of PCa in humans, as the loss of *PTEN* is one of the first genetic rearrangements observed in localized cases [7]. Animal breeding and housing were carried out at the animal facility of the CRCHUQ-Université Laval and followed the guidelines and regulations of the Canadian Council on Animal Care (CCAC). Mice were kept at 22°C and followed a 12 h light:12 h dark cycle.

4.2. Animal experiments and RNA extraction

Similar settings as Pihlajamaa and colleagues [8] used to study the androgen response were followed. In essence, since mice produce essentially all their sex hormones via their gonads [9], 16 WT and 14 PCa-developing male mice of at least 24 weeks of age were castrated to ensure

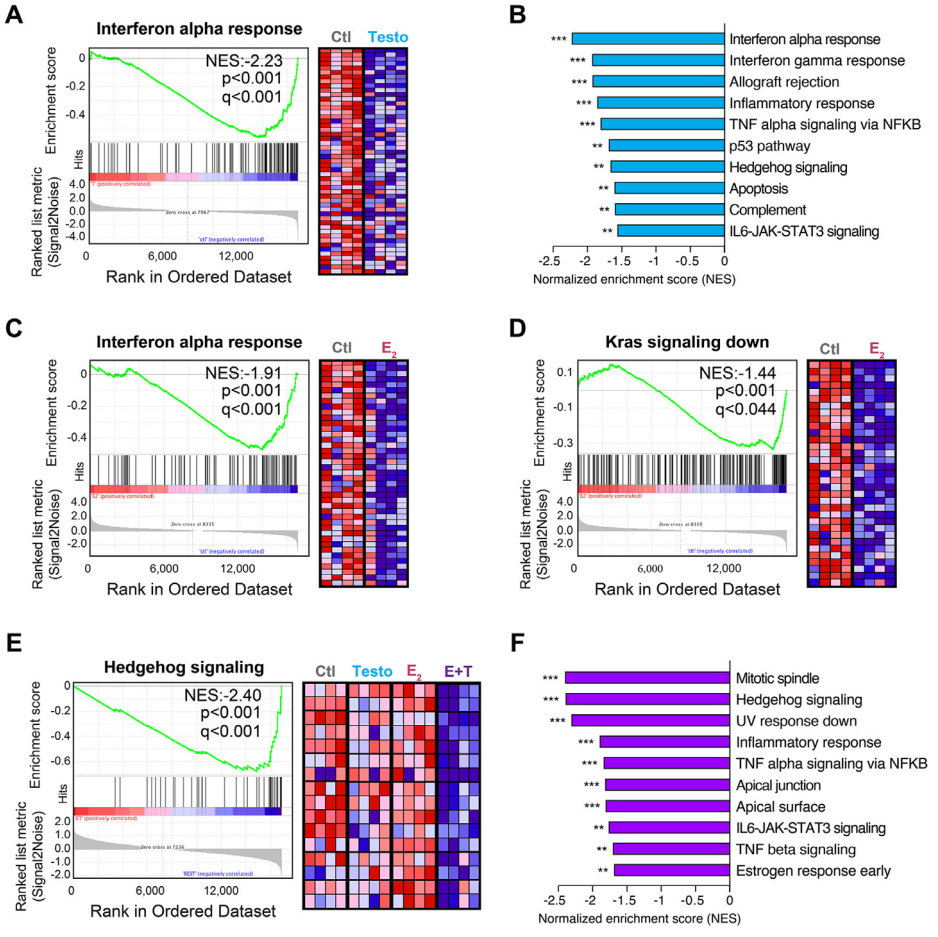

Fig. 1. Androgens and estrogens downregulate pathways notably linked to immunity in the wild-type mouse prostate. (**A**) Gene Set Enrichment Analysis (GSEA) diagrams and heatmaps of the interferon alpha response gene set following treatment with testosterone (Testo) compared to vehicle (Ctl). (**B**) Normalized enrichment scores (NES) of significantly downregulated gene set following treatment with Testo compared to Ctl. (**C, D**) GSEA diagrams and heatmaps of the interferon alpha response (**C**) and Kras signaling down (**D**) gene sets following treatment with estradiol (E_2) compared to Ctl. (**E**) GSEA diagrams and heatmaps of the Hedgehog signaling gene set following co-treatment with estradiol and testosterone (E + T) compared to all other individual treatments (Ctl, Testo, E_2). (**F**) Normalized enrichment scores (NES) of significantly downregulated gene sets following co-treatment with E + T compared to all other individual treatments. For each diagram and heatmap (**A, C-E**), only core genes of the gene sets are shown, and NES, *p*-values and false discovery rates (FDR, *q*-values) are indicated. For NES histograms (**B, F**), only the 10 significant gene sets with the lowest NES are shown and $^{*}q < 0.05$, $^{**}q < 0.01$ and $^{***}q < 0.001$.

circulating steroid depletion. After three days, they received subcutaneous injections of either vehicle (vegetable oil mixed with ethanol 96 %, 1:10 v/v), 17β-estradiol (E_2; 30 µg/g of mouse weight; Sigma), testosterone (30 µg/g of mouse weight), or a combination of both hormones. There were at least three mice per treatment group. After 24 h, mice were sacrificed and their prostates were harvested, flash-frozen and kept at −80 °C until RNA extraction. Each prostate was considered as one biological sample. As such, they were individually grinded in liquid nitrogen with a mortar and pestle laid on dry ice. The resulting powder from each tissue was collected separately before being directly used for RNA extraction (using the QIAGEN RNeasy Mini Kit and following the protocol given by the company). The eluted RNA of each sample was kept at −80 °C until sequencing.

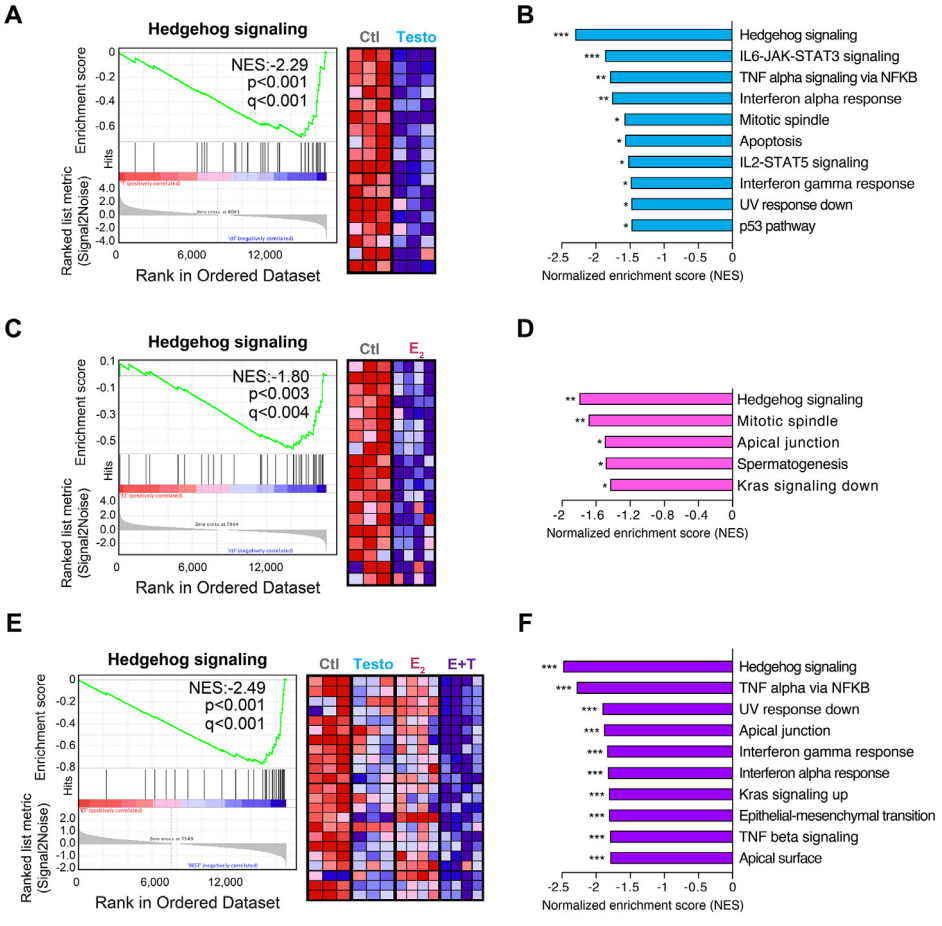

Fig. 2. Murine prostate transcriptome is reprogrammed by sex hormones in a tumoral context, with a predominant downregulation of the Hedgehog signaling. (**A, C, E**) Gene Set Enrichment Analysis (GSEA) diagrams and heatmaps of the Hedgehog signaling gene set following treatment with testosterone (Testo) compared to vehicle (Ctl) (**A**), treatment with estradiol (E_2) compared to Ctl (**C**), and co-treatment with estradiol and testosterone (E + T) compared to other treatments (Ctl, Testo, E_2) (**E**). (**B, D, F**) Normalized enrichment scores (NES) of significantly downregulated gene sets following treatment with Testo compared to Ctl (**B**), treatment with E_2 compared to Ctl (**D**), and co-treatment with E + T compared to other treatments (**F**). For each diagram and heatmap (**A, C, E**), only core genes of the gene sets are shown, and NES, *p*-values and false discovery rates (FDR, *q*-values) are indicated. For NES histograms (**B, D, F**), only the 10 significant pathways with the lowest NES are shown and $^{*}q < 0.05$, $^{**}q < 0.01$ and $^{***}q < 0.001$.

4.3. Sequencing

RNA quality was first verified on TapeStation 2200 bioanalyzer (Agilent), and all samples displayed an excellent RNA integrity number (RINe > 8). mRNA library preparation was completed with the NEBNext Ultra II Directional RNA library prep kit following the manufacturer's protocol, and samples were sequenced with a NovaSeq 6000 at the Next Generation Sequencing Platform of the CRCHUQ-UL (paired-end, 100 bp sequence length).

4.4. Bulk mRNA-seq data analysis

Raw sequencing data were acquired in fastq format. Depth ranged from 20 to 42.1 M reads after trimming, except one sample having only 7.9 M reads (Normal_ET-1). FastQC and MultiQC

were first used for data quality control [10,11]. Low-quality reads and adaptor content were removed with Trim_Galore/Cutadapt on default parameters [12,13], before using FastQC and MultiQC again to reconfirm data quality. Kallisto was employed on default parameters to pseudo-align the trimmed sequences to the Gencode vM25 murine reference transcriptome [14]. In this manuscript, the GSEA tool [5] was used to evaluate the downregulated pathways induced by androgens and estrogens in both normal and tumoral murine prostate, using Broad Institute's mouse orthologs hallmark gene sets collection and permutating gene set a 1,000 times, while keeping the other parameters at default.

Limitations

Since *in vivo* models were used to produce this dataset, the impacts of castration and hormonal treatments could also have disrupted the feedback loop of the hypothalamo-pituitary-gonadal axis; as such, the observed transcriptomic changes could be partly caused by the modulation of other hormones than androgens or estrogens (such as LH of FSH). Moreover, the related research article associated with this dataset focused on the estrogen receptor ERα, but E_2 can have other targets in prostate cells, such as GPER (G-protein coupled estrogen receptor), which can induce signaling cascades that could also explain some of the transcriptomic changes observed. GSEA analyses presented herein are based on mouse and human gene orthology; thus, depending on the quality of the MSigDB conversion, some genes could have been wrongly considered as orthologs, which could alter result interpretation. Finally, one sample from the dataset has low sequencing depth (Normal_ET-1), but it can be discarded without disrupting the robustness of the analyses since there are four replicates for this condition.

Ethics Statement

All animal work performed to generate this dataset was approved beforehand by Université Laval Research and Ethic Animal Committee (CHU-22-1206) in accordance to ARRIVE guidelines, and followed the general guidelines of the Canadian Council on Animal Care (CCAC) as well as the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No 8023, revised 1978). To study the prostate's transcriptome following sex hormone treatments, all selected mice were male given that this gland is specific to biological males in this species.

CRediT Author Statement

Camille Lafront: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft, Review & Editing, Visualization. **Lucas Germain:** Software, Validation, Formal analysis, Investigation, Data curation. **Étienne Audet-Walsh:** Conceptualization, Formal analysis, Writing – Original Draft, Review & Editing, Visualization, Supervision, Project administration, Funding acquisition

Data Availability

The reprogrammation of the estrogen and androgen responses in murine prostate cancer (Reference data) (Gene Expression Omnibus (GEO)).

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Declaration of Competing 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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