Dynamic prostate cancer transcriptome analysis delineates the trajectory to disease progression

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Comprehensive genomic studies have delineated key driver mutations linked to disease progression for most cancers. However, corresponding transcriptional changes remain largely elusive because of the bias associated with cross-study analysis. Here, we overcome these hurdles and generate a comprehensive prostate cancer transcriptome atlas that describes the roadmap to tumor progression in a qualitative and quantitative manner. Most cancers follow a uniform trajectory characterized by upregulation of polycomb-repressive-complex-2, G2-M checkpoints, and M2 macrophage polarization. Using patient-derived xenograft models, we functionally validate our observations and add single-cell resolution. Thereby, we show that tumor progression occurs through transcriptional adaption rather than a selection of pre-existing cancer cell clusters. Moreover, we determine at the single-cell level how inhibition of EZH2 - the top upregulated gene along the trajectory - reverts tumor progression and macrophage polarization. Finally, a user-friendly web-resource is provided enabling the investigation of dynamic transcriptional perturbations linked to disease progression.
Many decades of research have established the fundamental understanding of cancer as an anarchistic proliferation and dissemination of cells caused by acquired mutations in key driver genes\(^1\). During the past decade, the most common cancer types have been extensively characterized for alterations in the tumor DNA sequence\(^2\). While these studies have been initially conducted on primary cancer tissues, more recent clinical studies have also included biopsies from metastatic disease\(^2\)–\(^9\). Because of the binary nature of DNA sequence alterations (mutated versus non-mutated), mutation frequencies can be readily compared across studies and enable the nomination of drivers intimately linked to disease progression and outcome\(^10,11\). That said, the plethora of complex genetic alterations largely complicates a quantitative assessment of the transformed phenotype.

The assessment of gene expression may provide a more complete and quantitative measure of the biological processes related to disease progression. Most transcriptomic studies have been thus far conducted on primary tumors\(^12,13\). However, multiple efforts have been dedicated in recent years to the characterization of metastatic disease for a few tumor types, including prostate cancer, opening the possibility to assess transcriptional changes along with disease progression in a systematic manner\(^11,14–17\).

Nevertheless, this approach requires the accurate integration of multiple datasets across studies to overcome the issue of introducing dataset-specific features, often referred to as batch effects. The substantial amount of nonbiological artifacts introduced both by RNA-sequencing (RNA-seq) library generation techniques and by the exploitation of different quantification algorithms are among the difficulties that can emerge in the attempt of nominating a trajectory of prostate cancer disease progression by inferring dynamic transcriptional changes from a large integrated cohort.

Here, we provide a framework to overcome these issues and enable the accurate quantitative integration of RNA-seq data from over 1000 clinical tissues ranging from normal prostate tissue to primary prostate cancer (PNPCa) and metastatic castration-resistant (CR) prostate cancer (CRPC). The harmonized Prostate Cancer Transcriptome Atlas provides a unique resource to mine transcriptional changes related to different disease stages. Using this resource, we characterize the trajectory to disease progression and functionally validate our findings in patient-derived xenograft (PDX) models at the single-cell level. Finally, we show how our Prostate Cancer Transcriptome Atlas can infer or validate new therapeutic avenues for cancer patients.

**Results**

**Generation of the Prostate Cancer Transcriptome Atlas.** To nominate gene-expression changes related to disease progression, we re-processed and integrated high-throughput transcriptional datasets from 13 different studies, constituting thus far the most comprehensive compendium of the disease (Supplementary Fig. 1A and Supplementary Data 1)\(^11,16–24\). The resulting principal component analysis (PCA) showed that samples’ position at a given disease stage largely overlapped with another regardless of their origin. In contrast, samples from distinct disease stages differed in localization (Fig. 1a). An appreciable “batch effect” related to the hybrid capture sequencing (HCS) technique was detected and subsequently corrected (Supplementary Fig. 1B).

Gene-set enrichment analysis (GSEA) of the first two principal components (PCs) revealed that PC1 correlated with enhanced proliferation, while PC2 anticorrelated with canonical AR signaling (Supplementary Fig. 1C, D). Moreover, PC3 separated cancers harboring truncal mutations in *SPOP* and *FOXA1* from the ones harboring gene fusions involving ETS family transcription factors (Supplementary Fig. 1E)\(^25–28\). Additional PCs accounting individually for <4% of the total variance did not reveal any association with tumor cell-specific features. Importantly, the stromal contribution was well represented by PC5 and to a much lesser extent associated with PC1–4 (Supplementary Fig. 1F–H and Supplementary Data 2). The latter indicates that the positioning of tissue samples in PC1–4 is only slightly influenced by the tumor purity.

**Trajectory analysis quantifies the path to disease progression.** We applied trajectory inference analysis to characterize disease progression. The approach identified the path to disease progression and assigned a pseudotime to each sample that describes the advancements along this specific path (Fig. 1b). Because PC3 was mainly influenced by truncal prostate cancer driver mutations, its addition to the trajectory inference analysis did not affect the assigned pseudotime (Supplementary Fig. 1I, J). Subsequently, we assessed corresponding gene-expression changes to the initial two-dimensional trajectory (Fig. 1c). Among the most upregulated genes, we noticed key genes encoding for chromatin remodelers, which mediate gene silencing during development, such as DNA methyltransferases (DNMTs) and members of the polycomb-repressive complex-2 (PRC2)\(^29\). Most importantly, the PRC2 member *EZH2* emerged as the top upregulated gene, corroborating its previously suggested role in disease progression (Fig. 1c and Supplementary Fig. 1K)\(^15,30,31\). Besides, among the most upregulated genes, we noted AR-regulated genes that promote G2–M cell cycle progression, while AR-regulated differentiation genes were suppressed, as expected (Fig. 1d)\(^32–34\).

The progression path indicates that most prostate cancers evolve from normal tissue by continuously increasing AR signaling (PC2). Then, under androgen deprivation therapy, the tumors progress to CRPC by increasing cell cycle genes and eventually dedifferentiate to AR-negative disease with or without neuroendocrine features (neuroendocrine prostate cancer (NEPC)) (Fig. 1e). Notably, the transcriptional changes correlated well with the protein level changes in an independent set of primary and CRPC samples (Fig. 1f)\(^35\). Because EZH2 protein quantification was not performed in this dataset, we ascertained its upregulation with disease progression on a tissue microarray (TMA) of 33 primary and matched CRPC samples (Supplementary Fig. 1L)\(^36\).

Next, we evaluated whether genomic alterations in driver genes correlate with disease progression. We noted a significant correlation of point mutations in *PIK3CA*, *TP53*, *FOXA1*, *KMT2C*, and *PTEN* with progression in primary tumors and *FOXA1* in the metastatic counterpart (Fig. 1g). In primary tumors, we also noticed a positive correlation with *MYC* copy number and an inverse correlation with deletions of *RB1*, *PTEN*, and *TP53*, as expected. In contrast, in CRPC/NEPC samples, only *RB1* loss seemed to correlate well with increased progression (Fig. 1h and Supplementary Fig. 1M, N).

We wondered if pseudotime would also predict survival in patients with metastatic disease. Indeed, increased pseudotime significantly correlated with overall survival (Fig. 1i). While loss-of-function mutations in *RB1* and *TP53* were also associated with poor survival, these alterations did not outcompete pseudotime in the multivariate analysis. Hence, pseudotime still reached significance when only *RB1* wild-type tumors were considered (Supplementary Fig. 1O, P). The data suggest that pseudotime assessment may be useful to predict patient survival in an advanced disease setting.

Finally, we assessed transcriptional changes in key immune pathways throughout tumor progression along the trajectory. It has been widely appreciated during recent years that cancer
growth is supported by changes in the tumor microenvironment, such as the polarization of macrophages from an M1—towards M2-like phenotype. Indeed, we noticed a potent down-regulation of pro-inflammatory M1 markers and an increased and continuous shift towards M2-associated pro-tumorigenic effectors (Fig. 1c, j and Supplementary Fig. 1Q–S). Interestingly, CD24—a potent “don’t eat me” signal for M1 macrophages—was associated with upregulation of key proteins related to polycomb complexes (EZH2, SUZ12, EED), DNA methylation (DNMT1, DNMT3A/B), and G2-M cell cycle progression (Fig. 2b).

Integration of prostate cancer models in the transcriptome analysis. We next set out to further functionally validate our findings related to disease progression in eight established human prostate cancer cell lines and six PDX models originating either from a surgically, carried off PNPCa or CRPC (LuCaP-23.1, LuCaP-35, LuCaP-78, LuCaP-145, and LuCaP-147). To this end, the transcriptional fingerprint of all models clustered towards the outer layer of the progression trajectory (Fig. 2a and Supplementary Fig. 2A, B).

As expected, the PCA positioning of cell lines and the PDX models along the trajectory was highly significantly associated with the originating disease stage and the dependence on androgens (Supplementary Fig. 2C). The hormone-naive (HN) PNPCa model was placed first, followed by the CRPC-derived models, positioned progressively according to their decreasing levels of AR dependency. Finally, we observe the AR-negative (PC3, DU-145) and neuroendocrine models (NCI-H660, LuCaP-145.2), which are located at the end of the route (Fig. 2a and Supplementary Fig. 2A, B).
Multiple CR sublines of cell lines and PDX models have been generated over the past decades, enabling us to further functionally validate the disease progression trajectory in an isogenic system. Indeed, we found that all sublines progressed on the trajectory (Fig. 2c and Supplementary Fig. 2D–F). Most notably, the LTL-331 PDX model displayed a gradual transcriptional progression from CRPCs and primary tumors. Polycomb-repressive complex-related genes highlighted in orange, cell cycle-related genes in green, immune response in light blue, and AR signaling in magenta. X-axis: Pearson’s correlation coefficient between mRNAs and pseudotime; Y-axis: the associated significance adjusted for false discovery rate (FDR) and expressed in the form of \(-10 \times \log(10 \text{FDR})\). See Source data file.

The ex vivo culture of prostate cancer cells has been traditionally a major challenge. That said, the adjustment of the 3D organoid culture system for prostate cancer has enabled the ex vivo culture of PDX- and pseudotime, and are depicted in the indicated color scale. 

**Single-cell resolution to the trajectory.** We performed single-cell RNA-seq (scRNA-seq) of most aforementioned PDX models in vivo to interrogate the individual cells’ distribution along the trajectory of disease progression. In each case, normal mouse stromal cells were identified and separated from human tumor cells (Fig. 3a and Supplementary Fig. 3A–D). When comparing the merged single-cell data with the previously generated bulk RNA-seq data, we noticed in each case an excellent concordance between the position of both data points on the PCA plot, suggesting that our single-cell data are sufficiently similar to allow the integration into the pan-prostate cancer transcriptome cohort (Fig. 3b and Supplementary Fig. 3E–H).

Subsequently, we interrogated each PDX for the existence of separate subpopulations using the Seurat workflow (Fig. 3a and Supplementary Fig. 3A–D) and integrated the data into the PCA plot (see “Methods” section). Overall, single cells of the various subpopulations within a given PDX model did not greatly differ in their position to the trajectory and displayed relatively little overlap across PDX models (Fig. 3b and Supplementary Fig. 3E–L). As expected, subpopulations in cell cycle progression (i.e., S and G2M phase) positioned higher on the trajectory (Fig. 3b and Supplementary Fig. 3E–P). That said, the PDX model LuCaP-35 showed a wider distribution of subpopulations along the trajectory with distinct features linked to the S and G2M phase (H1–3 versus H4, 6), respectively, raising the possibility of being composed of two major, biologically diverse tumor clones (Supplementary Fig. 3G, K, O).

Subsequently, we assessed if and how these subpopulations would evolve during progression to androgen independence. For this purpose, we took advantage of the LuCaP-147 PDX tumor model that quickly develops castration resistance and compared the single-cell transcriptional profiles before and after castration (Fig. 3c). Upon regrowth, there was no major difference in the position and abundance of previously identified subpopulations (Fig. 3d). Instead, we noticed a concordant shift along the trajectory for each of the clusters h1–7, which was characterized by a shutdown of canonical AR signaling and upregulation of pro-proliferative MYC target genes, among others (Fig. 3e, f). Altogether, the data suggest that resistance to castration in this setting occurs likely through reprogramming of...
the entire tumor cell population instead of a clonal selection of a particular cluster.

Subsequently, we wondered if the induction of resistance may be paralleled by changes in the tumor microenvironment. Indeed, after castration, we observed an increase in the abundance of tumor-associated macrophages that displayed a change in polarization from M1- to M2-like features (Fig. 3g, h). In line with this, we also observed a gradual reduction of inflammatory signatures—key features of M1 macrophages—in PDX models with increasing pseudotime along the trajectory (Fig. 3i). The results agree with the expression changes of M1- and M2-related transcripts along the trajectory of disease progression described earlier in Fig. 1. Taken together, the data illustrate how bulk transcriptional changes related to disease progression can help to shed light on the emergence of androgen-independent prostate cancer at the single-cell level.

Co-targeting AR and EZH2 delays tumor progression. Because EZH2 emerged as a top upregulated transcript within the trajectory of disease progression and had been shown to promote androgen independence, we set out to investigate if co-targeting AR and EZH2 may prevent or substantially delay disease progression. Indeed, we noted a dramatic change in the transcriptional output program of LNCaP cells when treated with the EZH2 protein inhibitor GSK126 under androgen-deprived conditions in charcoal-stripped serum (CSS) (Fig. 4a). Previously detected LNCaP subpopulations (h1–h8) formed a new subpopulation (h7), suggesting a nearly complete rewiring of transcription and upregulation of AR signaling, reduction of EZF-related cell cycle genes, and reversion of progression on the trajectory (Fig. 4b and Supplementary Fig. 4A–D). In line with this, we noticed a strong reduction in colony formation when androgen-dependent LNCaP, VCaP, and LAPC4 cells were subjected to CCS and treated with GSK126, while forced expression of EZH2 was sufficient to promote colony formation in the same setting (Supplementary Fig. 4E).

Next, we tested if our observations would also translate into an in vivo setting. For this purpose, we injected LNCaP cells into the
flank of immune-compromised mice and treated the emerging xenograft tumors with castration alone or in combination with 3 weeks of GSK126. In both cases, the tumors fully regressed. While the tumors of castrated mice regrew with a latency of around 4 weeks, GSK126 co-treated tumors took more than twice as much time to re-initiate tumor growth (Fig. 4c).

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We subsequently performed scRNA-seq on the tumors pre- and post castration to investigate transcriptional changes on tumor and stromal cell subpopulations. As noted previously for LuCaP-147, we found no major change in the tumor cell subpopulations (i.e., h1–6) that adapted to castration (Fig. 4d).

Because GSK126 treatment in vivo had been stopped for 3 months before harvesting the tumors, the transcriptional changes in the tumor cells appeared less striking than in the aforementioned cell culture setting (Fig. 4a, d). That said, we observed after GSK126 co-treatment a continuous relative increase in tumor cell numbers (Fig. 4c).

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We further assessed the interaction between LNCaP cells and macrophage-like THP1 cells in vitro. The supernatant from THP1/LNCaP cocultures in CSS promoted M2 polarization on THP1/LNCaP cells (Fig. 4i, j and Supplementary Fig. 5C, D). Importantly, xenograft-associated macrophages also continuously increased in numbers and displayed a shift towards M2-like polarization in tumors adapted to castration as previously observed (Fig. 4d, f, g). Strikingly, we found a pronounced relative reduction of preferentially M2-like macrophages in GSK126-pretreated tumors, suggesting that GSK126-mediated changes on the tumor microenvironment may have contributed as well to the delayed regrowth of LNCaP xenografts (Fig. 4h).

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Supplementary Fig. SSE-1). At the same time, GSK126 reverted M2 polarization (Fig. 4i, j). In line with this, GSK126 suppressed the induction of cytokines capable of M2 induction in LNCaP cells (i.e., CSF1, IL13, IL4; Fig. 4k and Supplementary Fig. S5j). Indeed, the supernatant of GSK-treated LNCaP cells was sufficient to revert CSS-induced polarization of human M2 macrophages (Fig. 4l and Supplementary Fig. S5k, l).

In aggregate, the data suggest a rationale for joint targeting of AR and EZH2 in prostate cancer because the latter reverts tumor cell progression towards a more androgen-dependent state and at the same time counteracts adaptive changes in macrophages and fibroblasts that are intimately linked to disease progression.

Discussion

In the present study, we combine transcriptional profiles of prostate cancers at various disease stages to a comprehensive Prostate Cancer Transcriptome Atlas with negligible study-level of the progression trajectory. Our results suggest that resistance to androgen deprivation may occur through transcriptional adaptation of tumor cells towards a more progressed state. In line with this, a recent study has proposed that prostate regeneration (a process that shares many molecular features with regeneration within 4 weeks after castration.

Fig. 3 Single-cell resolution to the trajectory. a Dimensionality reduction of single-cell distribution of LuCaP-147 PDX model in vivo using Uniform Manifold Approximation and Projection (UMAP) and subsequent identification of cell clusters performed using Seurat workflow. Human (right) and mouse cells (left) are separated from each other. A total amount of 7 and 4 clusters could be identified for human and mouse cells, respectively. For the latter, we indicated the cells of origin corresponding to the various clusters on top. Inference of cell types was performed with SingleR through the exploitation of the ImmGen repository. For human cell clusters, we indicated the inferred cell cycle phase as predicted using Seurat. Human and murine cell clusters are depicted using different colors as indicated on top of the figure panel. b Projection of single-cell clusters on the PCA plot. The position of merged single-cell data corresponds to the one from bulk RNA-sequencing data. Please refer to the “Methods” section for detailed information on scRNA-seq data integration with bulk RNA-seq. Cell clusters are depicted using different colors as indicated on top of the figure panel. c LuCaP-147 xenografts regress and regrow within 4 weeks after castration. N = 5 independent experiments. Error bars indicate standard error. d Comparison of tumor single-cell clusters before (left) and after castration (right). Cell clusters are depicted using different colors as indicated on top of the figure panel. e Violin plot shows an increase in pseudotime of individual cells within the cell clusters after castration. To deal with drop-out events, the pseudotime inference was performed for each cell following imputation of missing genes using RMagic. Pre-castration (Pre-CX): red; post castration (Post-CX): blue. f Gene sets perturbed in LuCaP-147 xenografts’ single-cell clusters at regrowth (post castration) compared to pre-castration. Most hallmark gene sets are upregulated (red) or downregulated (blue) similarly. A marked downregulation of AR-responsive genes is noted. Differential expression for each cluster denoting the transcriptional changes occurring after castration was determined using the MAST algorithm. Subsequently, we determined the gene-set enrichments using Camera (pre-ranked). g Dimensionality reduction (UMAP) of murine macrophages (green) pre-castration (left) and post castration (right) highlights a notable increase in macrophage count at regrowth. h After castration, the percentage of infiltrated macrophage to tumor cell ratio increases. Pre-CX (red): 8.1%; post-CX (regrowth, blue): 17.1%. Statistical significance was computed using Pearson’s χ² test. See Source data file. i After castration, macrophages display more M2-like transcriptional features according to the macrophage polarization index, as determined by using Mac Spectrum. Significance levels (P values) were determined using Wilcoxon’s rank-sum test (two-tailed). Pre-CX: red; post-CX (Regrowth, blue). See Source data file. j Single samples gene-set enrichment analysis of inflammation-related pathways performed following reclustering of murine macrophages extracted from the corresponding single-cell RNA-seq experiments. Missing gene-expression values (drop-out events) for each cell were imputed using RMagic. With increasing pseudotime along the trajectory, macrophages of xenograft models display less active TNFA (dark gray) and inflammatory signaling (light gray). PNPCa xenografts were excluded from the analysis because of the limited number of infiltrated macrophages. Error bars indicate standard error.

early CRPC because it may prevent the dedifferentiation of cancer cells as an escape mechanism to AR-directed therapeutic interventions. In line with previous reports, we noticed along the trajectory a change of macrophage polarization from inflammatory M1 to pro-tumorigenic M2. Our findings further underscore the antitumor potential of pharmacologically re-educating macrophages towards M1. Castration was sufficient in our PDX models to induce a change toward M2 polarization after a relatively short period in line with previous reports, suggesting that therapeutic interventions per se may be at least in part the underlying cause. Importantly, we show in the same setting that inhibition of EZH2 protein substantially blocked the castration-induced polarization change towards M2, uncovering a thus far underappreciated role for EZH2 in macrophage polarization another rationale towards co-targeting AR and EZH2 in prostate cancer.

It is mostly unknown how disease progression in prostate cancer emerges at the single-cell level. Using a series of PDX models reflecting different progression stages from HN to AR-negative late-stage disease enabled the addition of single-cell resolution to the progression trajectory. Our results suggest that resistance to androgen deprivation may occur through transcriptional adaptation of tumor cells towards a more progressed state. In line with this, a recent study has proposed that prostate regeneration (a process that shares many molecular features with prostate cancer progression) is driven by nearly all persisting luminal cells, not just by rare stem cells. That said, in our study, we have used a relatively uniform xenograft tumor model that has been already derived from CRPC and thus adapt swiftly to castration in mice. Conceivably, resistance to androgen receptor inhibition over a longer period may also involve the selection of stem-cell-like subpopulations irrespective of the presence of genetic drivers of CRPC (e.g., AR amplification or point mutations).

We provide a web-based interface for the research community to facilitate the mining of the Prostate Cancer Transcriptome Atlas, called the PCAProfiler (https://www.pcaprofiler.com). Using this resource, we readily identify, for example, that a subpopulation of very advanced prostate cancer tissues expresses high levels of IL23A, a
Recently described to mediate castration resistance in prostate cancer. Interestingly, correlating the *IL23A* expression with genomic features in our webtool identifies a tight association of *IL23A* expression with gains and amplification of its receptor *IL23R*. Such insights may be important for patient selection/stratification for anti-IL23 targeting monoclonal antibodies under clinical development (i.e., NCT04458311).

The PCaProfiler will also allow the pseudotime annotation of new cancer transcriptomes. In a clinical trial setting, this information may enable identifying antitumor responses within a certain subset of patients with a given degree of disease progression. In a preclinical setting, the atlas may also help researchers to choose the corresponding model system that reflects the disease stage under investigation. Of note, in this...
regard, we have already annotated the pseudotime for the most frequently used prostate cancer cell lines (see PCaProfiler). Alternatively, the PCaProfiler may enable researchers to verify and optimize the ex vivo culture condition so that it best mirrors the in vivo setting.

In conclusion, we successfully merged the RNA-seq data from several prostate cancer studies, covering different disease stages. Based on that, we delineate the roadmap to prostate cancer progression in a qualitative and a quantitative manner. Furthermore, we also show how individual tumor cells can be tracked along the progression trajectory in response to pharmacological perturbations. Because the transcriptome data of advanced metastatic disease will become more readily available for other tumor types, the current study may serve as a blueprint for their analysis and exploitation.

Methods

Experimental model and subject details

Plasmids. The PhAGE-puro (Plasmid #118692) and the PhAGE-EZH2 (Plasmid #116738) were purchased from Addgene.

Cell lines. PC3, DU-145, 22rv1, MDA-MB-231, LAPC4, LNCaP, VCaP, and HEK 293T cell lines were purchased from ATCC (American Tissue Culture Collection) (Manassas, USA). The LAPC4 cell line was a gift from Prof. Helmut Klocker, the LNCaP-abl cell line was a gift from Prof. Myles Brown (DFCI, Boston), and the THP1 cell line was a gift from Prof. Saverio Minucci (IEO, Milan). The supernatant of all cell lines was routinely tested (twice per month) using the MycoAlert™ Mycoplasma Detection Kit (Catalog #: LT07-318, Lonza). All cell lines resulted negative for Mycoplasma infection.

Immunohistochemically staining. EZH2 protein abundance was analyzed on TMA, including matched primary and CRPC samples (University of Bern). All prostate cancer samples from human subjects were obtained under approval by the Ethics Committee of Northwestern and Central Switzerland (EKNZ, Nos. EK/1311 and EK/1314/2008). This is based on a retrospective study. Two prostate core needle biopsies were used to analyze benign prostate (n = 3 patients). Prostate cancer biopsies included in the TMA were taken during routine clinical treatment. Samples were selected based on the following inclusion criteria: (a) histologically diagnosed PCa, (b) tumor-containing biopsies available at HN and CR state, and (c) sufficient quality and amount of material, as evaluated by experienced pathologists (IPT). Castration resistance was defined as either biochemical progression (i.e., serum PSA progression according to the Prostate Cancer Clinical Trials Working Group criteria or clinical progression. A TMA comprising 112 matched HN/CR tissue specimens and including 107 transurethral resections and five distant metastases derived from 53 PCa patients was constructed. Briefly, tissue cylinders with a diameter of 1 mm were punched from the patient’s tissue blocks containing the specimens using the robotic precision instrument Grand Master TMA (3D Hitche). Tissue cylinders were placed in one recipient paraffin block. After the block construction was completed, an 8 μm section of the resulting TMA block was mounted to a microscope slide. Due to tissue loss, a common problem associated with TMA technology, 33 high-quality matched tissue samples of primary and CRPC remained after sectioning.
For EZH2 IHC, slides were analyzed with the Bond-III Automated Staining System (Leica) using manufactured reagents for the entire procedure. For antigen retrieval, slides were incubated for 60 min in citrate buffer at pH 8 at 98 °C. Thereafter, slides were incubated with a rabbit monoclonal antibody against EZH2 (D2C9, CST5246 from Cell Signaling) at the dilution of 1:500. Detections were performed using the detection refine DAB Kit (Leica). Immunohistochemical staining was evaluated as the percentage of tumor cells with nuclear positivity for EZH2 using Aperio ImageScope (Leica).

**Cell culture.** PCs, DU-145, 22V1, LAPC4, LNCaP, and THP1 cells were cultured in RPMI-1640 (21875-034, Life Technologies) supplemented with 10% FBS, 25 mg/ml gentamicin, 100 mg/ml streptomycin, and 1% penicillin/streptomycin at 37 °C.

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**PEI-mediated transfection and lentiviral infection.** Lentiviral production was carried out by polyethylenimine (PEI)-mediated transfection of the HEK 293T with PHAGE (Empty, Addgene 118692) and PHAGE-EZH2 (Addgene 116738) vectors. Briefly, The HEK 293T cells were seeded in a 10 cm culture dish (4 × 10^6 cells/plate) and incubated overnight at 37 °C in a 5% CO2 humidified atmosphere. After 24 h, the vector plasmid (PHAGE or PHAGE-EZH2; 3 μg), the packaging plasmid (pCMV-dR8.2; 2.7 μg), and the envelope plasmid (pSVG-G; 0.7 μg) were mixed in Opti-MEM™ 1 Reduced Serum Medium (300 μl/10 cm culture dish, Thermo Fisher 31985070) with 1.25 mM PEI (Sigma-Aldrich, 919012) solution (ratio μl PEI:μg DNA: 1:1). The DNA/PEI mixtures were incubated at room temperature for 15 min. The DNA/PEI mixture was then added to the supernatant of the HEK 293T cell. Forty-eight hours after the transfection, the viral supernatants were collected and filtered through a 0.45 μm filter. The LNCaP cell line was incubated with viral supernatant and 8 μg/ml Polybrene (H9268, Sigma) for 72 h and then selected with 2 μg/ml puromycin (P8833, Sigma) for 2 weeks. Western blotting was used to verify EZH2 protein abundance.

**Animal experiments.** All animal experiments were carried out according to protocol approved by the Swiss Veterinary Authority/Board (TI-42-2018 and TI-10-2010) and received approval by the ethical committee of the Institute of Oncology Research. All in vivo studies used 6–8-week-old male NRG (NOD:Ragtm1Il2rgtm1, NOD rag gamma) mice.

**Housing conditions.** Before experimental procedures, mice were housed in individually ventilated cages, maintained at room temperature (20–22 °C) and a 12 h light cycle. Groups of five mice were kept in individual cages of ~465 cm². The cages were sealed, autoclaved before use, and used in a “Sealsafe” rack (Techniplast) with a 0.2-μm aerosol bacteria barrier vent. All manipulation of the cages (e.g., to replace bedding) occurred in a cage changing station (CCS, Techniplast), designed to maintain air filtration in a sterile airflow environment. For experimental procedures, mice were housed in groups of 4–5 mice in ~355 cm² filter-topped cages, on racks in a specified pathogen-free barrier facility. Cages and filters were autoclaved before use, and experimental procedures and manipulation of the cages occurred in a sterile laminar flow hood (Skan AG). PDX LuCaP-147, LuCaP-145.2, LuCaP-145.1, LuCaP-147v2, and VCAp were provided by Dr. Diya Corey. The PDX series has been established by subcutaneous transplantation of tumor tissue of patients with metastatic prostate cancer tumors, from 1991 to 2005. Tissue collection for research was approved by the University of Washington Human Subjects Division IRB, which approved all informed consents that were used for tissue collection. (IRB #39071, IR). Dr. Marjana Kruthof-de Julio provided PNPCa. The established PDX was originated from a patient who presented with primary PCa on March 2014. The patient included in the study provided written informed consent (Cantonal Ethical approval KEK 06/ 2015 ‘Colony formation assay in DHT-free medium. VCAp (5 × 10^5 cells/well), LAPC4 (2.5 × 10^5 cells/well), LNCaP (2.5 × 10^5 cells/well), or LNCaP-overexpressing EZH2 were maintained in triplicate in 6-well plates and incubated overnight at 37 °C. When the cells were attached to the plate and formed a confluent layer, the medium was replaced with 10% CSS medium (DHT-free medium) with without 1 μM GSK126 and kept in culture until the formation of the colonies (4–6 weeks). The medium/treatment was weekly replaced. At the end time point, the cells were gently washed with PBS, fixed with 0.1% crystal violet, and 20% of EtOH for 30 min, and then wash out with water. The images of colonies were acquired using the Fusion Solo IV LBR system and the quantification of colonies was performed by the ImageJ software.

**Antibodies and Western Blot Analysis.** The primary antibodies used were: anti-GAPDH (sc-7727, Santa Cruz), anti-AR (sc-7505, Santa Cruz), anti-DNMT3A (sc-397704, Santa Cruz), anti-AR (sc-6267, BD Transduction), anti-DNMT3A (sc-50332S, Cell Signaling Technology), anti-EED (sc-365769, Santa Cruz), anti-EZH2 (sc-4996, BD Transduction Laboratories), anti-H3K27m3 (sc-35625, Cell Signaling Technology), and anti-PLK1 (sc-376756, Biolegend).

**Tumor tissues (25–30 mg) or cellular pellets were lysates with RIPA buffer supplemented with cocktail phosphatase inhibitors (4906845001, Roche) and proteases inhibitors (5892953001, Roche). Protein concentration was determined by BCA reagent (A52255, Thermo Fisher Scientific); 30–50 μg of whole protein lysate was separated on 8–12% SDS–polyacrylamide gels and transferred onto PVDF membrane (88518, Thermo Fisher Scientific). The membranes were blocked with 5% milk in Tris-buffered saline with Tween-20 (TBST) for 30 min at RT, incubated overnight at 4 °C with primary antibodies, and incubated for 1 h at RT with secondary antibodies (anti-rabbit IgG HRP W401B and anti-mouse IgG HRP W402B, Promega). The protein bands were visualized using the western bright imaging reagent (K-12482-D20, Advanta) and quantified using the Fusion Solo IV LBR system.
each spot. The signaling of each spot was normalized using the average signaling of two different array-specific positive controls. To calculate the average fold changes (FCs), the signaling of each normalized spot was compared with the average normalized signal of the corresponding DHT-treated condition.

**RT-qPCR analysis.** According to the manufacturer’s guidelines, the RNA extraction was performed from a cellular pellet of THP1-derived macrophages using an RNasey Kit (74106, Qiagen). Quantitative reverse transcription PCR (RT-qPCR) was carried out using KAPA SYBR® FAST One-Step (KK4600, Sigma) following the manufacturer’s protocol. The primer sequences were obtained from PrimerBank (http://pga.mgh.harvard.edu/primerbank/index.html). The list of the primer is reported in Supplementary Data 3. Actin was used as a housekeeping gene. The qPCR analysis was performed using the 2^(-ΔΔCt) method.

**Flow cytometry analysis.** For phenotype analysis, isolated THP1-derived macrophages or human M2-like macrophages were suspended in PBS containing 1% fetal calf serum and then stained for 30–45 min at RT with a cocktail of marker antibodies. For staining anti-CD14-BV650, anti-CD80-PE, anti-CD163-PECy7, and anti-CD206-BV510 antibodies (eBiosci) were used. Samples were acquired on a BD LSR-Fortessa flow cytometry (BD Biosciences). Data were analyzed using the FlowJo software. FACS gating/sorting strategy is indicated in Supplementary Fig. 6.

**In vitro differentiation of THP1-derived macrophages.** The THP1 cells were polarized to M1-like macrophages in RPMI medium, 10% FBS, and 100 ng/ml of phorbol-12-myristate-13-acetate (PMA) (Sigma, P1585) for 24 h following stimulation with 10 ng/ml interferon-γ (300-02, PeproTech) and 10 ng/ml of lipopolysaccharide (Saccharide, Sigma, 916374) for 48 h. The unpolarized THP1-M0 were derived from THP1 cells stimulated for 48 h only with PMA RPMI medium and 0% FBS. After stimulation, the TH1-M0 or the THP1-M1 cells were maintained in CSS, CSS 1 nM DHT, or CSS 1 µM GS Kill26 fresh medium or cocultured with LNCAP cells.

**In vitro differentiation of peripheral blood mononuclear cell-derived macrophages.** Buffy coat was mixed 1:2 with PBS. The mixture was then added 3:1 to Ficol gradient (Invitrogen, 17-1440-03) and spun down at 1500 r.p.m. for 25 min at RT (w/o brakes). The leukocyte ring was collected and washed with PBS. Cells were then resuspended in 0.5% bovine serum albumin in PBS containing anti-CD14 microbeads (130-050-201, Miltenyi Biotech) and purified for positive selection using LS MACS column system (130-042-401, Miltenyi Biotech). The purity of CD14+ cells was analyzed by FACS using an anti-CD14-BV650 antibody (eBiosci). The CD14+ cells were polarized to M2-like macrophages in RPMI medium, 10% FBS, 50 ng/ml granulocytemacrophage colony-stimulating factor (R&D Systems, 216-GMP), 20 ng/ml interleukin-13 (200-13, PeproTech), and 20 ng/ml of IL4 (200-04, PeproTech) for 7 days. In the experiments with CSS, CSS 1 nM DHT, or CSS 1 µM GS Kill26 fresh medium and LNCAP-CM, the CD14+ cells were polarized to M2-like macrophages using the same cocktail of cytokines.

**RNA extraction for RNA-seq analysis.** According to the manufacturer’s guide lines, the RNA extraction was performed from PDX’s frozen fragment (25–30 mg) of cellular pellet using RNeasy Kit (74106, Qiagen). The RNAs were processed using the NEB Next Ultra II Directional Library Prep Kit for Illumina (E7765, NEB) and sequenced on the Illumina NextSeq 500 with single-end, 75-bp paired-long reads.

**Single-cell isolation for scRNA-seq.** To perform scRNA-seq PDX tumor tissue, they were dissociated into single cells as described above (see section “Ex vivo culture of PDX”). After resuspension in PBS, single-cell suspensions were loaded into a 10x Chromium Controller (10x Genomics, Pleasanton, CA, USA), aiming for 10,000–50,000 cells, with the Chromium Nex Gen Single Cell 3’ v3.1 Reagent Kit (PN-1000121, 10x Genomics), according to the manufacturer’s instructions.

**RNA-seq data processing.** Sequencing of xenografts and 2D and 3D cultures. We retrieved bulk RNA-seq data for cellular models of prostate cancer from various available datasets and extended these by performing bulk RNA-seq of several prostate cancer Xenograft models (i.e., PNPc; LuCaP-78, LuCaP-23, LuCaP-35, LuCaP-145; LNCAP), and their derived 3D cultures. Additional sequencing was performed for 2D cultures of LNCAP, LNCAP-all, LACPC, and VCaP cells (see “Data availability” section).

**Prostate Cancer Transcriptome Atlas.** To build an integrated resource of transcriptional features representing all stages of prostate cancer progression, we collected raw sequencing data from a large panel of independent datasets. We gathered raw data for 1223 clinical samples (1104 excluding technical replicates, 1044 excluding multiple metastatic sites derived from the same individual). The resulting integrated cohort is representative of various stages of disease progression, namely, normal prostate specimens (n = 174), primary tumors (n = 714), CRPCs (n = 316), and CRPCs showing features of neuroendocrine trans-differentiation (n = 19). Raw sequencing files were retrieved from following sources: (1) Gene Tissue Expression Dataset (2) The Cancer Genome Atlas (TCGA); (3) atlas of RNA-sequencing profiles of normal human tissues (GSE120795); (4) integrative epigenetic taxonomy of PNPCs (GSE120741); (5) prognostic markers in locally advanced lymph node-negative prostate cancer (PRINAA7449); (6) the long noncoding RNA landscape of NEPC and its clinical implications (PRJER121992); (7) the human prostate cancer event (APEC); and (8) the complete set of 1223 samples (Supplementary Fig. S1B) showed that the largest samples sharing the same batch-effect-related features. A PCA analysis performed on identifying the transcriptional features endowed with the highest variance across all samples, was a very useful tool to detect relevant batch effects. When the latter are overwhelming, they are likely to appear among the top PCs and cluster together samples showing the same batch-effect-related features. A PCA was performed on the complete set of 1223 samples (Supplementary Fig. S1B) showing that the largest source of batch effects was associated with the HCS technique, while no relevant differences could be clearly associated with the dataset of origin. Only two of the CRPC datasets (phs000915 and phs000673) contained samples sequenced using HCS, and for several of those, matched technical replicates sequenced using PolyA+ were also available. This allowed us to assess and remove technology-associated bias in gene expression (ComBat algorithm, sva package v3.38.0, PolyA+ training set only). This way, for PCA representation, we avoid the selection of genes that are possibly affected by the sequencing technique, despite the correction we had already performed above-mentioned number (4 × 500 = 2000).

**RNA-seq data processing of clinical samples.** The overall quality of sequencing reads was evaluated using FastQC (v0.11.9). Sequence alignments to the reference human genome (GRCh38) were performed using STAR (v2.6.1c) in two-pass mode, to significantly increase sensitivity to novel splice junctions compared to the regular single mapping. Briefly, in the two-pass mapping procedure, reads are mapped twice in the first pass, the novel junctions are detected and inserted into the genome; in the second pass, all reads are re-mapped annotated (from the GTF file) and novel (detected in the first pass) junctions. In particular, gene expression was quantified at the gene level in the second pass by the comprehensive annotations made available by Gencode (v29 GTF File). Strand-specific information was not maintained to avoid technical differences between stranded and unstranded libraries. Samples were adjusted for library size and normalized with the variance stabilizing transformation (vst) in the R statistical environment using DESeq2 (v2.8.1) pipeline. When performing differential expression analysis between groups, we applied the embedded independent filtering procedure to exclude genes that were not expressed at appreciable levels in most of the samples considered. If not otherwise specified, all GSEA analyses were performed using the limma (v3.46.0) package (Camera, use. ranks set to TRUE). Gene-set collections were retrieved either from the Molecular Signature Database (MsigDB) or from previous publications (NE-Score and AR/NE-Score)76. V values were calculated for mapping testing using the false discovery rate (FDR) procedure, with the significance threshold set to 0.05. In addition, GSEA significance was logarithmically transformed in form of −log10(p-adjusted), with a bold intercept (x = 13.01) indicating the FDR threshold depicted in the corresponding plots.

**Batch-effect correction and PCA.** In the process of integrating different datasets from a variety of sources, we verified that batch effects did not overwhelm the biological signal. Batch effects may derive not only from differences across datasets but also may be a consequence of a different sequencing technique (PolyA+; TotalRNA; HCS) or originate from other unknown sources. We aimed at specifically removing technical batches rather than real biological variation and tried to preserve biological differences that may be of interest to a different dataset. To assess tumor purity, tumor grade/stage, or other. PCA, by rather than real biological variation and tried to preserve biological differences that may be consequent of a different PSA level, age, tumor grade/stage, or other. PCA, by rather than real biological variation and tried to preserve biological differences that may be consequent of a different PSA level, age, tumor grade/stage, or other. PCA, by
and NEPCs is due to a real biological signal and not consequent to an unwanted dataset-specific batch effect.

Integration and validation of additional bulk RNA-seq samples and pseudotime inference.

We developed a method to include new prostate tumor samples in our current analysis by starting from raw counts, which allows the computation of pseudotime and PCs without modifying the original data and plots. Ideally, RNA-seq should be quantified using the sample genome (hg38) and references used for the current study (Genomic V29). Performed sequential passage of a new sample of interest, raw counts will be merged with the ones composing our full set. The obtained numeric matrix (the original matrix + 1 extra sample of interest) undergoes the same normalization and processing steps up to the computation of the PCA. Here, coordinates may slightly differ from the original ones, due to the addition of a new sample that might exert a small effect on the global re-normalization of all samples. To address this behavior, we apply a machine learning-based approach (glmnet package, v1.4.1) that generates at runtime three elastic net models, one for each of the top 3 PCs, and train them to predict the error between the original coordinates and ones that are recomputed following the addition of the extra sample of interest. Hence, we apply these models to adjust the computed PC1, PC2, and PC3 coordinates of the extra sample, which can now be added to the PCA plot and pseudotime can be determined using slingshot.

Trajectory analysis.

Trajectory and pseudotime inference are frequently used in scRNA-seq data analysis to model developmental trajectories through smoothed curves following dimension reduction and clustering. Here, we applied one of these tools, slingshot (v1.6.0), to infer progression-associated trajectory and pseudotime from our integrated set of bulk RNA-seq samples. We selected slingshot because of its capability to also determine branches along the trajectory if any. PCA positioning (PC1–PC2) of the indicated data set is used for mapping the samples with respect to the normal tissue cluster. The analysis was performed using 1106 samples, discarding all technical replicates, in order not to overweight some samples and influence the computation of the trajectory. Metastatic lesions from the same individual but localized in different organs were admitted for this analysis. Subsequently, we could associate a pseudotime for each sample, ranging from 0 to 250 (Fig. 1b).

Correlation of genes and pathways to pseudotime.

Having defined a unique pseudotime value for each sample, we computed the correlation between pseudotime and mRNA expression for each gene. For this purpose, we used Pearson’s correlation over Spearman’s because we aimed at identifying the strength of the linear relationship between gene expression and pseudotime. However, to be more robust to outliers, we opted for ten times repeated leave one-third out procedure. Precisely, we randomly selected ten subsets composed of 66% of the samples and computed correlation coefficients between pseudotime and expression of each gene in all subsets. Finally, we averaged these values and ranked them according to their correlation coefficient to pseudotime. Subsequently, using this ranking we applied Camera to perform GSEA procedure (use.rank = TRUE) and determined which gene sets were mostly directly or inversely associated with pseudotime (Supplementary Fig. S1F).

Correlation of mRNA expression and protein abundances.

Proteomics data were retrieved from the Proteomics Identifier Database (PRIDE: projects PXD009868, PXD003340, PXD003452, PXD003515, PXD004132, PXD003615, PXD003636, see “Data availability” section). The dataset includes 28 gland-confined prostate tumors and 8 adjacent non-malignant prostate tissues obtained from radical prostatectomy procedures, plus 22 bone metastatic prostate tumors obtained from patients operated to relieve spinal cord compression. To compute the correlation between mRNA expression and protein abundance, we first computed, for each gene, the average FC (log2) between CRPC and primary tumors based on mRNA expression. Then, the same was applied to the proteomics data to obtain for each protein a log FC representing differential abundance between CRPCs and primary tumors. For protein/mRNA correlation purposes, we discarded all genes that had not been evaluated in the proteomic data. Finally, we used Pearson’s method to evaluate the strength of correlation and the associated statistical significance.

Retrieval of genetic information and correlation with progression.

Matched genetic information respective to mutations and copy-number status could be retrieved for 763 samples through cBioportal. Samples for which this information was available are indicated in Supplementary Data 1. To determine associations between mutations and tumor progression, for each gene we compared the pseudotime of mutant versus wild-type samples, by performing statistical testing using Wilcoxon’s rank-sum test (two-tailed). Genes were ordered according to their FDR-adjusted P values and analyses were performed separately in primary and CRPC + NEPC tumors, to determine the relative contribution of mutations at various stages of disease progression. We only screened for genes being mutated in more than five individuals (Supplementary Fig. S1L). To determine associations between copy-number alterations and tumor progression, for each gene we used a value of either –2 (homozygous deletion), –1 (heterozygous deletion), 0 (wild type), 1 (gain), 2 (amplification), and subsequently computed Pearson’s correlation between these values and pseudotime. We restricted this last analysis to genes being frequently deleted or amplified in prostate samples, namely, MYC, AR, RBL, PTEN, and TP53 (Fig. 1e). The above-described analyses were performed discarding technical replicates. Metastatic lesions from the same individual but localized in different organs were admitted for this analysis.

Quantity of immune infiltrates and correlation with progression.

Quantification of immune infiltrates for all samples in our cohort was inferred from transcriptomic data using CibersortX by using the default signature matrix “LM22” to deconvolve 22 immune cell subsets from bulk RNA-seq data (absolute quantification mode). The abundantly inferred immune populations was correlated to pseudotime using the same strategy applied to correlate gene expression and pseudotime. We opted for ten times repeated leave one-third out procedure. Precisely, we randomly selected ten subsets composed of 66% of the samples and computed correlation coefficients between pseudotime and each immune population in all subsets. Finally, we averaged these values and ranked them according to their correlation coefficient to pseudotime. Pearson’s correlation-associated P values were corrected for multiple testing using the FDR.

Macrophage polarization index.

The macrophage polarization index, indicating polarization towards M1 or M2 phenotype, was computed for all bulk RNA samples in our cohort using MacSpectrum v0.41.

scRNA-seq data processing.

Quantification of gene expression. Fastq files were generated by demultiplexing raw data using cellranger (v3.1.0). To make single-cell gene-expression quantification more comparable to those of bulk RNA-seq, we generated a custom genome with cellranger, using the very same reference (GRCh38.p12) and analyses (Genocode v29) we had used for STAR when performing bulk RNA-seq analysis. To discriminate between human and murine cells that may infiltrate the tumors in the in vivo setting, we created a Mouse-Human reference, by creating a hybrid genome (GRCh38.p12 + GRCMa4p6) and hybrid gene-annotations (Genocode v29 and M25, for human and mouse genes, respectively). To avoid conflicts, mouse genomic coordinates were preceded by a prefix (i.e., mm_chr1, mm_chr2, etc.). Subsequently, cellranger was used to quantify gene expression in the form of an h5 filtered matrix where Ensemble gene IDs are used as identifiers.

Data filtering and clustering. Expression quantification files were imported in R statistical environment using Seurat (v3.1.5) package. We discarded individual cells from our data matrix by using two filtering procedures: first, we aimed at detecting transcriptome outliers, and second, we looked for putative doublets, which were discarded. Briefly, we computed per-cell quality control metrics using scater (v1.16.1). The total amount of mitochondrial and ribosomal gene expression was quantified for both human and mouse cells. The number of genes being detected per cell, the total amount of reads per cell and the mitochondrial and ribosomal fraction of the transcriptome were used to determine the skewness-adjusted multivariate outlyingness for each cell (robustbase v0.93-6). Outliers were detected by median absolute deviation and removed at both tails. Counts were then normalized (Seurat::NormalizeData, method = LogNormalize, scale.factor = 1000) and the top 2000 most variable features were selected (Seurat::FindVariableFeatures, method = vtst). Data were then scaled (Seurat::ScaleData). Gene and PCA was performed up to the top 50 components (Seurat::RunPCA). Subsequently, we identified and eliminated putative doublets using DoubletFinder (v2.0.3). Having identified outliers and doublets, we removed them from the original count data and went through the preprocessing step again (i.e., normalization, scaling, and pca reduction). We proceeded to the determination of the k nearest neighbors of each cell and the construction of a shared nearest-neighbor (SNN) graph (Seurat::FindNeighbors), then we identified clusters using the SNN modularity optimization-based clustering strategy (Seurat::FindClusters, resolution = 0.5). Finally, we performed Umap dimensionality reduction on the first ten PCs, annotated the previously identified clusters, and generated plots accordingly.

Identification of cell cycle phase and cell type.

We retrieved the list of cell cycle markers, and subdivided it into markers of G2/M phase or S phase, according to Seurat’s annotations. We then used this information to infer the cell cycle phase in our samples (Seurat::CellCycleScoring). Murine cells could be clearly distinguished from human cancer cells, because of the intrinsic differences that could be easily spotted owing to the alignment and quantification performed using a hybrid human-mouse genome. Murine cell types were identified using SingleR (v1.2.4), using ImmGen repository.

Dealing with drop-out events.

Drop-out events are very frequent in the single-cell experiment performed using 10x Chromium technology. To address these issues, we applied Markov affinity-based graph imputation of cells (RMagic v2.0.3).

Differential expression analysis and gene-set enrichment. Differential expression analysis was performed between different cell clusters and between clusters subjected to different treatment conditions (Seurat::Findmarkers) using a hurdle model tailored to scRNA-seq data (MAST method). Genes were subsequently ranked for log FC, and the Camera algorithm (pre-ranked) was used, we looked for putative doublets, which were discarded. Cell-specific gene-set enrichments were determined using single-sample GSEA, computed using gene-expression values of each cell following RMagic imputation.
Macrophase polarization index of macrophages. The macrophase polarization index, indicating polarization towards M1 or M2 phenotypes was computed for all cells being identified as macrophages from SingleR analysis (https://mcspectrum.ucconn.edu).

Macrophase reclustering. We could identify a sustained number of murine macrophages infiltrating all xenograft models, except for NPCIa cells. We isolated them and performed a cell-type-specific analysis by repeating all previously described processing steps (i.e., normalization, scaling, and pca reduction). Drop-out events were addressed using RMagic, and cell-specific enrichments were computed using a single-sample GSEA.

Integration of scRNA-seq with bulk RNA samples, PCA, and pseudotime inference. Single-cell experiments can be easily integrated with bulk RNA experiments by simply summing up together gene counts for all individual cells into one metadata element. This has proven to be comparable in terms of pseudotime inference and PCA positioning, as scRNA-seq and bulk RNA-seq experiments performed on the same cells are overimposable to each other. The same applies for the integration of single-cell-derived clusters, provided that the number of cells composed each cluster is not so critically low that the number of drop-out events results in a matrix composed of too many missing genes. If this is the case, or if just a single cell is to be integrated into the analysis, we suggest running RMagic to deal with the drop-out events, and then simply proceed as previously described.

Additional resources PCaProFinder. We provide a resource for the research community endowed with a web-based interface to facilitate the mining of the Prostate Cancer Transcriptome Atlas, called the PCaProfiler (https://www.pcaprofiler.com). Using this resource, scientists can easily interrogate the atlas, recapitulate the findings shown in this study, and extend these by exploring correlations between genes of interest and prostate cancer progression. PCaProfiler will allow integration and pseudotime inference of new cancer transcripts that the user can directly upload, compute, and visualize on the server. All results can be downloaded and re-uploaded to PCaProfiler when needed. Preloaded are PCA positioning and pseudotime inferences of the cell line, xenografts, and organoid models, as well as single-cell clusters and additional transcriptional datasets not included in the current study (i.e., PRER25542, ESCAPE Trial). PCaProfiler will be updated frequently with new data as new samples are being released or under specific requests.

Quantification and statistical analysis. Quantification methods and statistical analysis methods were described and referenced in the respective “Method details” subsection. If not specified, all statistical tests were corrected for multiple comparisons using the FDR correction method.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability The bulk RNA-seq data generated in this study have been deposited in the EMBL-EBI database under accession code E-MTAB-9930. The single-cell RNA-seq data generated in this study for LuCaP PDX models and INCaP cells have been deposited in the EMBL-EBI database under accession code E-MTAB-9993. The publicly available RNA-seq data used in this study are available in GEO (Gene Expression Omnibus), SRA (Short Read Archive), and EMBL-EBI databases under accession codes GSE12079525, GSE12074119, GSE11843522, GSE12607821, PRJNA47744924, PRERP210929, and E-MTAB-9656. The ProteomesX data used in this study are available in the PRIDE database under accession codes PXD009868, PXD003430, PXD003452, PXD003515, PXD004132, PXD003615, and PXD003636. A minimum dataset to reproduce our findings containing vst-normalized expression data, along with its annotations, was made available (Zenodo repository, https://doi.org/10.5281/zenodo.5546618). All the software used for the analyses is described and referenced in the respective “Method details” subsections. All gene sets used for enrichment analyses were retrieved from the Molecular Signature Database (MsigDB). Source data are provided with this paper.

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Competing interests
M.A.R. is listed as a co-inventor on the US and International patents in the diagnostic and therapeutic fields of ETS gene fusion prostate cancers (Harvard and the University of Michigan) and SPOP mutations (Weill Cornell Medicine). J.-P.P.T. has received funding for the venue of scientific conferences from Astellas, MSD, and Janssen/Cilag. S.G. (past 3 years): honoraria—Janssen Cilag; consulting or advisory role (including IDMC)—Astellas Pharma, Amgen, Roche, Pfizer, AAA International, Janssen, Innocrin Pharma Inst, Sanoft, Bayer, Orion Pharma GmbH, Clovis Oncology, Menarini Silicon Biosystems, Tolero Pharmaceuticals, and MSD; patents, royalties, and other intellectual property—method for biomarker WO2009138392; travel grant—ProteoMediX; other relationship—Aranda. The remaining authors declare no competing interests.

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