

RESEARCH ARTICLE

Differentially Expressed Genes and Signature Pathways of Human Prostate Cancer

Jennifer S. Myers¹, Ariana K. von Lersner¹, Charles J. Robbins¹, Qing-Xiang Amy Sang^{1,2*}

1 Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida, United States of America, **2** Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida, United States of America

* qxsang@chem.fsu.edu



OPEN ACCESS

Citation: Myers JS, von Lersner AK, Robbins CJ, Sang Q-XA (2015) Differentially Expressed Genes and Signature Pathways of Human Prostate Cancer. PLoS ONE 10(12): e0145322. doi:10.1371/journal.pone.0145322

Editor: Jian Cao, Stony Brook University, UNITED STATES

Received: October 14, 2015

Accepted: December 2, 2015

Published: December 18, 2015

Copyright: © 2015 Myers et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was in part supported by the Leslie N. Wilson-Delores Auzenne Graduate Assistantship for Minorities awarded to JSM by the Florida State University Graduate School, the Research Experience Program of Women in Math, Science, and Engineering of Florida State University to AKVL, and grants from the Florida State University and an Endowed Chair Professorship in Cancer Research from anonymous donors to QXAS. The funders had no role in study design, data collection

Abstract

Genomic technologies including microarrays and next-generation sequencing have enabled the generation of molecular signatures of prostate cancer. Lists of differentially expressed genes between malignant and non-malignant states are thought to be fertile sources of putative prostate cancer biomarkers. However such lists of differentially expressed genes can be highly variable for multiple reasons. As such, looking at differential expression in the context of gene sets and pathways has been more robust. Using next-generation genome sequencing data from The Cancer Genome Atlas, differential gene expression between age- and stage- matched human prostate tumors and non-malignant samples was assessed and used to craft a pathway signature of prostate cancer. Up- and down-regulated genes were assigned to pathways composed of curated groups of related genes from multiple databases. The significance of these pathways was then evaluated according to the number of differentially expressed genes found in the pathway and their position within the pathway using Gene Set Enrichment Analysis and Signaling Pathway Impact Analysis. The “transforming growth factor-beta signaling” and “Ran regulation of mitotic spindle formation” pathways were strongly associated with prostate cancer. Several other significant pathways confirm reported findings from microarray data that suggest actin cytoskeleton regulation, cell cycle, mitogen-activated protein kinase signaling, and calcium signaling are also altered in prostate cancer. Thus we have demonstrated feasibility of pathway analysis and identified an underexplored area (Ran) for investigation in prostate cancer pathogenesis.

Introduction

Prostate cancer is the second most diagnosed cancer among American men, with over 220,000 new cases predicted in 2015 [1]. Prostate-specific antigen (PSA) has been the cornerstone of prostate cancer screening for decades. However PSA is not an ideal biomarker and widespread use of PSA-screening is falling out of favor [2–4]. Reliance on PSA screening is problematic because false positives result from benign prostatic hyperplasia or prostatitis and because PSA fails to discriminate indolent disease, leading to overdiagnosis. The expansion of genomic and

and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abbreviations: PSA, prostate-specific antigen; DEGs, differentially expressed genes; MAPK, mitogen activated protein kinase; TGF- β , transforming growth factor-beta; TCGA, The Cancer Genome Atlas; FDR, false discovery rate; PANTHER, Protein ANalysis THrough Evolutionary Relationships; GO, Gene Ontology; GSEA, Gene Set Enrichment Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; SPIA, Signaling Pathway Impact Analysis; ES, enrichment score; NES, normalized enrichment score; pNDE, probability of overrepresentation; pPERT, probability of perturbation.

proteomic technology and methodology has improved the characterization of tumor biology, driving the search for more accurate cancer biomarkers. Gene and protein expression differences between normal and malignant prostate tissues have been well documented and serve as a pool for putative diagnostic, prognostic, and risk stratification biomarkers [5–24]. Gene mutations, epigenetic changes, and microRNA expression changes that occur in cancer initiation and progression have also been studied with the goal of biomarker discovery [25–29]. Yet there remain several substantial obstacles in biomarker implementation. Low reproducibility across laboratories, differences in experimental platforms and techniques, the inherent heterogeneity of prostate cancer, and insignificant clinical utility or small gains in sensitivity and specificity beyond PSA hampers the identification, validation, and implementation of biomarkers [30–35].

Previous work has focused on the selection and validation of individual genes as biomarkers. Yet the heterogeneity of prostate cancer makes it extremely unlikely to find a single gene that is a representative marker [36]. Screening panels formed by the combination of multiple genes have been used to increase predictive power for cancer detection, recurrence, relapse, and survival beyond the use of PSA or Gleason score alone [37–40]. The success of the biomarker panel approach is evidenced by the commercial launch of several screening tests which have found clinical usefulness: ProMark [41], Oncotype DX [42], Prolaris [43], and Decipher [44]. These panels may be pulled from molecular classifications studies that use differential expression to craft a signature for cancer.

However molecular classifications and gene signatures are not always stable in the sense that multiple signatures can be found for cancers. Large discrepancies between lists of differentially expressed genes (DEGs) from microarray data have been highlighted [45]. In some cases the overlap between microarray datasets was as low as 5% [46]. So for each set of DEGs, a different signature could be found. Thus biomarkers selected from these lists would perform with varying degrees of success. Taking the list of DEGs and correlating them to a prognostic marker may generate a more useful putative biomarker pool because then only genes correlated with prognosis would comprise the molecular signature. However, Ein-Dor *et al.* showed that in breast cancer, there was no single, unique set of genes that predicted survival because altering the patient population could produce multiple sets of genes of equal prognostic ability in predicting survival [33]. Furthermore, correlation with survival was not required for prognostic ability [33]. So it is likely that many panels exclude a number of other genes that could be potential biomarkers because the panel was derived from one body of samples (although it may be large) and considered only strongest correlations.

An alternative approach is pathway-based analysis. In pathway analysis, a collection of related genes from the same pathway or network of interaction is assessed instead of examining a group of potentially unrelated genes that optimize sensitivity and selectivity of diagnosis or prognosis. There is increased overlap between data at the pathway level compared to overlap between lists of DEGs [46, 47]. Pathway analysis does not neglect the cooperative nature of genes and considers that oftentimes genes involved in the same process are often deregulated together. By looking at the pathway, minor variations in instrumentation or method are less likely to impact results, leading to more consistent results across different sets of data [48]. Thus the pathway approach yields more robust results, improves disease classification, and may reveal novel insights about a disease [49–51]. One type of pathway analysis starts with a differentially expressed gene and correlates the expression of genes involved in the same pathway or similar process with a particular diagnostic or prognostic outcome [52–54]. A similar iteration starts with a pathway of known importance in cancer initiation or progression and evaluates the prognostic power of its individual components. This has been done for the mitogen-activated protein kinase (MAPK) pathway [55], Akt [56], mTOR pathway [57, 58], Toll-like receptor signaling pathway [59], and other oncogene signatures [60].

Table 1. Prostate cancer patient clinical information from TCGA.

Characteristics	Samples (n = 225)	Tumor (n = 173)	Non-Malignant (n = 52)	Fisher's Exact Test P-value
Age				
< 65	155	121	34	0.609
≥ 65	70	52	18	
Pathological T stage				
T1	0	0	0	0.649
T2	113	84	29	
T3	103	82	21	
T4	8	6	2	
Unspecified	1	1	0	
Race				
White	92	50	42	0.701
Black	7	3	4	
Unspecified	126	120	6	
Ethnicity				
Not Hispanic	96	51	45	
Unspecified	129	122	7	
Gleason Score				
≤ 6	24	19	5	0.00168
7	129	89	40	
8–10	72	65	7	

doi:10.1371/journal.pone.0145322.t001

In this paper, comprehensive gene expression in human prostate cancer was characterized using an unbiased pathway approach. Next generation sequencing was used to obtain a profile of the differences in RNA expression between human tumors and non-malignant tissue from patients. Pathway analysis included Gene Set Enrichment Analysis and Signaling Pathway Impact Analysis. Two pathways were significantly associated with human prostate tumors—“Ran regulation of mitotic spindle formation” pathway and “transforming growth factor-beta (TGF-β) signaling” pathway.

Materials and Methods

RNA sequencing data

Level 3 de-identified data for prostate cancer samples and all available non-malignant samples from these prostate cancer patients was downloaded from The Cancer Genome Atlas (TCGA) data portal (<https://tcga-data.nci.nih.gov>). Level 3 describes data that has been processed and aggregated to give gene expression signals for a sample. For each sample, the data contains expression counts for up to 20,531 coding and non-coding RNA transcripts plus clinical information such as age, stage, Gleason score, PSA level, and race/ethnicity. Before analysis, tumor and non-malignant samples were randomly pulled to achieve an age- and stage-matched pool of 225 samples (S1 Table). A total of 173 prostate cancer samples and 52 non-malignant samples from 204 unique patients were analyzed. The patient clinical information is presented in Table 1.

Differential Gene Expression

The R programming environment (version 3.1.2) [61] was used to process raw data, perform statistical calculations, and perform differential expression analysis. After age- and stage-

matching, 393 transcripts were removed because they lacked expression in the 225 samples comprising the dataset. The RNA counts for the remaining 20,138 transcripts were rounded to the nearest whole number and compiled into a matrix to build the dataset. The magnitude of expression changes relative to non-malignant samples was also calculated by taking the base 2 logarithm of the tumor/non-malignant mean expression ratio. For genes with no expression in either the tumor or non-malignant samples, the \log_2 fold changes were adjusted by adding one to each mean and then calculating the ratio. All \log_2 values quoted are values after any such adjustments. Negative fold changes indicated down-regulation in tumor samples whereas positive values indicated up-regulation. The R package DESeq2 (version 1.6.3) [62] was used to identify DEGs in the TCGA patient RNA data. The computing was done on the Florida State University High Performance Computing Cluster. DESeq2 returned a P-value determined by Wald statistics and an adjusted P-value (Q-value) to correct for multiple comparisons testing using the Benjamini-Hochberg method to determine the false discovery rate (FDR). DEGs were defined as genes different with a FDR less than 1% ($Q < 0.01$).

To evaluate the significance of the identified DEGs, analyses were conducted to search for overrepresented pathways, gene set enrichment, and signaling pathway impact. First, overrepresented elements were identified among the DEGs. The Protein ANalysis THrough Evolutionary Relationships (PANTHER) Classification System and analysis tools were used to categorize DEGs by PANTHER protein class, Gene Ontology (GO) Molecular Function, and GO Biological Process to then determine if any of these classes or GO terms were overrepresented [63]. The PANTHER Overrepresentation Test (release 20150430) was used to search the data against the PANTHER database (PANTHER version 10.0 Released 2015-05-15) and the GO database (Released 2015-05-09) to identify either protein classes or GO annotations overrepresented in our data when compared to a reference human genome. P-values were adjusted using a Bonferroni correction.

Pathway Analysis

Gene Set Enrichment Analysis (GSEA) [64] was used to identify groups of genes enriched in either the tumor or non-malignant condition. The GSEA analysis tool (version 2.2.0) was downloaded from the Broad Institute website (<http://www.broadinstitute.org/gsea/index.jsp>). Curated gene sets of BioCarta and Reactome pathways were downloaded from the Broad Institute's Molecular Signatures Database. An additional gene set was constructed from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [65]. Pathways with the least relevance to prostate cancer were excluded. The KEGG pathways included in the analysis are listed in the Supporting Information (S2 Table). The entire RNA expression count matrix was loaded into the GSEA application without limiting the input to only DEGs. Both small (< 5 genes) and large (> 500 genes) gene sets were excluded from the analysis.

Signaling Pathway Impact Analysis (SPIA) was used to assess the importance of enriched pathways in terms of their impact and ability to activate or inhibit a pathway [66]. SPIA analysis was accomplished using the R package "SPIA" (version 2.18.0) [67]. Entrez IDs, \log_2 fold changes, and Q-values for all genes were compiled. The differential expression cut-off used in the SPIA algorithm was based on the FDR-adjusted Q-value. The analysis was run using the same tailored list of pathways as used in GSEA (S2 Table) and updated versions of these pathways were download prior to running the analysis (accessed 7/29/2015).

Results

Using a 1% FDR ($Q < 0.01$), DESeq2 analysis marked 11,115 genes and transcripts as statistically different between tumor samples and non-malignant samples in our TCGA dataset (S3

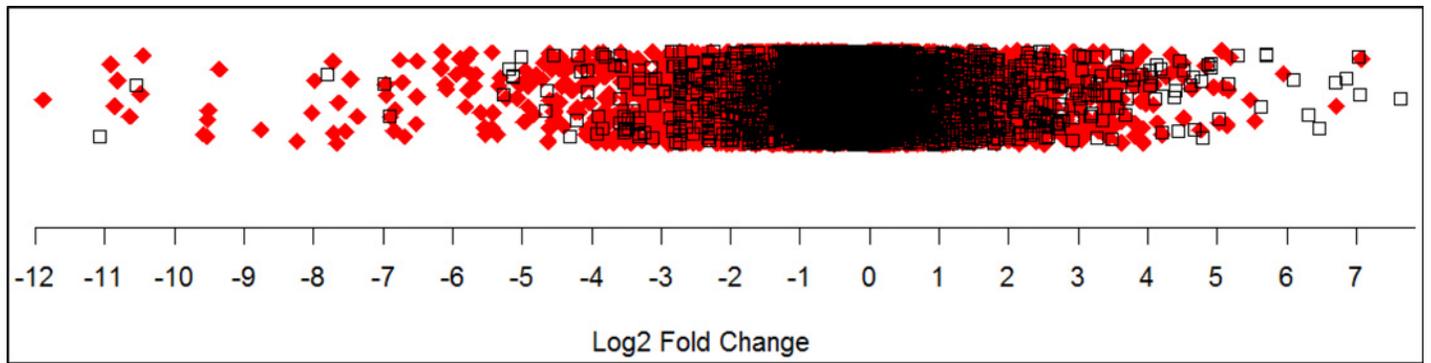


Fig 1. Magnitude of gene expression differences between tumor and non-malignant human prostate cancer samples. In this one-dimensional scatter plot the magnitude of gene expression changes represented by \log_2 fold ratios are shown. Each point represents a gene or transcript. Significantly differentially expressed genes and transcripts are shown as solid red diamonds.

doi:10.1371/journal.pone.0145322.g001

and S4 Tables). This covers 55% of the genes and transcripts sequenced. The number of down-regulated genes and transcripts totaled 5,379 and the number of up-regulated genes and transcripts totaled 5,736. Overall the largest changes observed were in the down-regulation of genes and transcripts (Fig 1). The magnitude of the up-regulation of genes and transcripts was smaller than the magnitude of down-regulated genes and the range of expression was also smaller. The twenty most down-regulated and the twenty most up-regulated genes are presented in Table 2 and Table 3.

Table 2. Twenty largest decreases in RNA expression between prostate tumor and non-malignant TCGA samples.

Gene Symbol	Name	Log ₂ Fold Change	P-value	Q-value
<i>WFDC9</i>	Protein WFDC9	-11.89	1.98E-04	4.30E-04
<i>DEFB125</i>	Beta-defensin 125	-10.91	4.30E-04	8.89E-04
<i>EDDM3B</i>	Epididymal secretory protein E3-beta	-10.85	4.64E-09	1.96E-08
<i>PAEP</i>	Glycodelin	-10.82	3.47E-16	3.78E-15
<i>SEMG2</i>	Semenogelin-2	-10.64	1.98E-63	2.36E-60
<i>PATE4</i>	Prostate and testis expressed protein 4	-10.48	2.26E-55	1.54E-52
<i>EDDM3A</i>	Epididymal secretory protein E3-alpha	-10.45	5.77E-13	4.05E-12
<i>CRISP1</i>	Cysteine-rich secretory protein 1	-9.58	1.17E-25	5.02E-24
<i>PATE1</i>	Prostate and testis expressed protein 1	-9.53	3.27E-27	1.76E-25
<i>DEFB127</i>	Beta-defensin 127	-9.52	1.06E-04	2.41E-04
<i>AQP2</i>	Aquaporin-2	-9.50	1.94E-57	1.69E-54
<i>TMEM114</i>	Transmembrane protein 114	-9.35	1.19E-15	1.21E-14
<i>GRXCR1</i>	Glutaredoxin domain-containing cysteine-rich protein 1	-8.75	5.95E-19	9.64E-18
<i>SPINT3</i>	Kunitz-type protease inhibitor 3	-8.23	2.11E-24	7.48E-23
<i>CLDN2</i>	Claudin-2	-8.02	2.11E-75	6.72E-72
<i>SULT2A1</i>	Bile salt sulfotransferase	-7.98	9.41E-20	1.70E-18
<i>SPINK2</i>	Serine protease inhibitor Kazal-type 2	-7.71	5.75E-71	8.46E-68
<i>POU3F3</i>	POU domain, class 3, transcription factor 3	-7.70	4.68E-17	5.77E-16
<i>LCN1</i>	Lipocalin-1	-7.66	4.18E-08	1.56E-07
<i>PATE3</i>	Prostate and testis expressed protein 3	-7.63	3.33E-25	1.32E-23

Log₂ fold change describes malignant expression relative to non-malignant expression. P-value is determined by DESeq2 using Wald Statistics and Q-value is the false discovery rate-adjusted P-value.

doi:10.1371/journal.pone.0145322.t002

Table 3. Twenty largest increases in RNA expression between prostate tumor and non-malignant TCGA samples.

Gene Symbol	Name	Log ₂ Fold Change	P-value	Q-value
<i>ANKRD30A</i>	Ankyrin repeat domain-containing protein 30A	7.08	5.95E-10	2.82E-09
<i>FEZF2</i>	Fez family zinc finger protein 2	6.71	1.89E-06	5.59E-06
<i>C6orf10</i>	Uncharacterized protein C6orf10	5.96	2.59E-06	7.52E-06
<i>FOXP1</i>	Forkhead box protein G1	5.54	2.53E-04	5.41E-04
<i>GC</i>	Vitamin D-binding protein	5.47	4.70E-04	9.67E-04
<i>VAX1</i>	Ventral anterior homeobox 1	5.19	3.83E-12	2.41E-11
<i>SSX2</i>	Protein SSX2	5.16	4.52E-03	7.92E-03
<i>FGB</i>	Fibrinogen beta chain	5.14	1.52E-03	2.88E-03
<i>SLC45A2</i>	Membrane-associated transporter protein	5.09	1.10E-51	5.99E-49
<i>SPINK1</i>	Pancreatic secretory trypsin inhibitor	5.07	3.29E-12	2.08E-11
<i>HOXC12</i>	Homeobox protein Hox-C12	5.03	1.44E-07	4.96E-07
<i>SCN1A</i>	Sodium channel protein type 1 subunit alpha	4.96	5.38E-03	9.31E-03
<i>LOC284661</i>	Uncharacterized non-coding RNA	4.84	4.89E-06	1.36E-05
<i>TFDP3</i>	Transcription factor Dp family member 3	4.76	2.00E-03	3.72E-03
<i>B3GNT6</i>	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6	4.64	5.49E-22	1.40E-20
<i>FOXB2</i>	Forkhead box protein B2	4.52	2.14E-18	3.19E-17
<i>NR2E1</i>	Nuclear receptor subfamily 2 group E member 1	4.51	1.21E-15	1.23E-14
<i>XAGE1E</i>	X antigen family, member 1E	4.51	4.00E-03	7.07E-03
<i>TBX10</i>	T-box transcription factor TBX10	4.43	6.47E-17	7.81E-16

Log₂ fold change describes malignant expression relative to non-malignant expression. P-value is determined by DESeq2 using Wald Statistics and Q-value is the false discovery rate-adjusted P-value.

doi:10.1371/journal.pone.0145322.t003

Classification and Overrepresentation Analysis

The 11,115 DEGs were grouped according to PANTHER protein class, GO Molecular Function and GO Biological Process annotations. A total of 6,254 DEGs had either PANTHER protein class, GO Biological Process, or GO Molecular Function annotations and were further classified. Grouping by protein class and GO Biological Process categories proved to be the most informative (Fig 2). The complete classifications can be found in the Supporting Information (S5 Table). The DEGs represent a wide spectrum of protein classes involved in a broad array of processes. The “Nucleic Acid Binding” PANTHER protein class includes both RNA and DNA binding proteins, nucleases, and helicases. The “Transcription Factor” protein class is sub-categorized by structural motif and also contains cofactors and nuclear hormone receptors. Proteases and phosphatases are found within the “Hydrolase” protein class. The types of “Receptor” included are protein kinase receptors, nuclear hormone receptors, cytokine receptors, ligand-gated ion channels, and G-protein coupled receptors. The “Enzyme Modulator” category features G protein, kinase, phosphatase, and protease modulators. Interestingly, the categories were generally not predominantly populated by down-regulated or up-regulated genes or transcripts. For all protein classes except the “Nucleic Acid Binding” class, DEGs were evenly distributed across tumor and non-malignant samples. In the “Nucleic Acid Binding” protein class, there were nearly one and half times as many up-regulated genes as down-regulated. The abundance of nucleic acid binding genes suggests altered transcriptional activity in tumor samples.

The two most abundant GO Biological Process groups—“Metabolic Process” and “Cellular Process”—are not surprising because these contains genes are involved in the most basic of life processes. In fact, metabolic changes have been widely documented in tumors [68–70]. The

increased energetic and biosynthetic needs of proliferating cancer cells are often met through metabolic dysregulation [71–73]. The heading “Metabolic Process” includes carbohydrate metabolism, cellular amino acid metabolism, lipid metabolism, nucleobase-containing compound metabolism, protein metabolism, and the tricarboxylic acid cycle. “Cellular Process” includes cell-cell signaling, cell cycle, growth and proliferation, cell component movement, and cytokinesis. “Biological Regulation” includes the regulation of apoptosis, metabolism, cell cycle, translation, catalytic activity, and homeostasis. The category “Developmental Process” incorporates system, ectoderm, mesoderm, and endoderm development, as well as cell differentiation, death, anatomical structure morphogenesis, embryo development, sex determination, and pattern specification processes. “Localization” refers to general transport proteins and specific protein and RNA localization processes.

PANTHER’s overrepresentation statistic was used to calculate the probability that the highly populated protein classes and GO groupings among the DEGs would occur by random chance. Indeed, many of the most abundant categories are overrepresented in the data when compared to a reference genome (Table 4). The three most abundant protein classes—“Nucleic Acid Binding”, “Transcription Factor”, and “Hydrolase”—were enriched along with the classes “Transferase” and “Transporter”. The five most populated GO Biological Processes were also enriched: “Metabolic Process”, “Cellular Process”, “Biological Regulation”, “Localization”, and “Developmental Process”. The “Multicellular Organism Process”, “Biological Adhesion”, “Cellular Component Organization or Biogenesis”, and “Immune System Process” GO Biological Processes were also enriched. Finally, five of the top six GO Molecular Functions were enriched: “Binding”, “Catalytic Activity”, “Nucleic Acid Binding Transcription Factor Activity”, “Transporter Activity”, and “Structural Molecule Activity”.

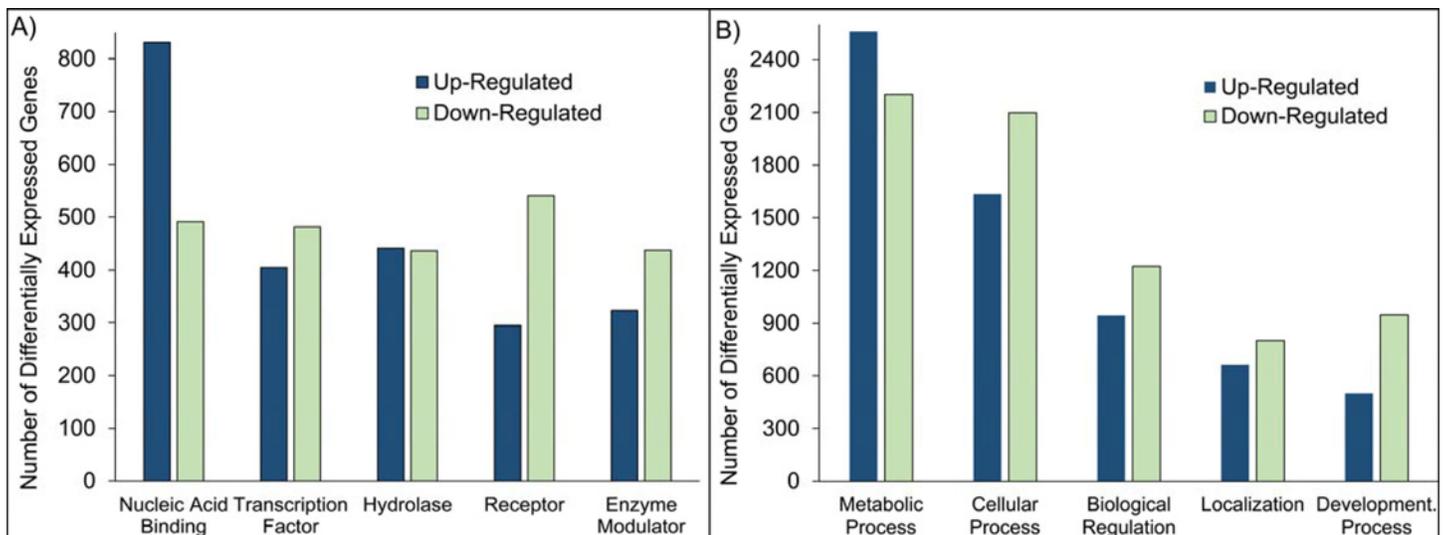


Fig 2. Functional Classification of Differentially Expressed Genes in Human Prostate Cancer According to PANTHER Protein Class (A) and Biological Process Gene Ontology Terms (B). (A) “Nucleic Acid Binding” includes RNA and DNA binding, nucleases, and helicases. “Transcription Factor” includes zinc finger, helix-turn-helix, high mobility group box, basic helix-loop-helix, and basic leucine zipper transcription factors; cofactors; and nuclear hormone receptors. “Hydrolase” refers to proteases, phosphatases, esterases, lipases, deaminases, phosphodiesterases, glycosidases, deacetylases, pyrophosphatases, glucosidases, galactosidases, and amylases. “Receptor” includes protein kinase receptors, nuclear hormone receptors, cytokine receptors, ligand-gated ion channels, and G-protein coupled receptors. “Enzyme Modulator” includes G protein, kinase, phosphatase, and protease modulators. (B) “Metabolic Process” features carbohydrate, cellular amino acid, lipid, protein, and nucleobase-containing compound metabolism; and the tricarboxylic acid cycle. “Cellular Process” categories are cell-cell signaling, cell cycle, growth and proliferation, cell component movement, and cytokinesis. “Biological Regulation” includes the regulation of apoptosis, metabolism, cell cycle, translation, catalytic activity, and homeostasis. “Developmental Process” categories are system, ectoderm, mesoderm, and endoderm development; cell differentiation; death; anatomical structure morphogenesis; embryo development; sex determination; and pattern specification processes. “Localization” includes transport proteins, protein and RNA localization processes.

doi:10.1371/journal.pone.0145322.g002

Table 4. Overrepresented PANTHER protein class and GO ontology categories in TCGA data from malignant and non-malignant prostate.

	P-value
PANTHER Protein Class	
RNA binding protein (Nucleic Acid Binding)	9.08E-05
Ribosomal protein (Nucleic Acid Binding)	4.67E-04
Transcription factor	2.04E-02
Transferase	3.41E-04
Hydrolase	5.75E-04
Transporter	3.65E-03
GO-Biological Process	
Sensory perception of chemical stimulus (Multicellular Organism Process)	2.96E-10
Protein metabolic process (Metabolic Process)	6.82E-09
Nucleobase-containing compound metabolic process (Metabolic Process)	1.64E-08
RNA metabolic process (Metabolic Process)	6.87E-06
Nervous system development (Developmental Process)	1.48E-03
Cellular protein modification process (Metabolic Process)	1.90E-03
Translation (Metabolic Process)	2.81E-03
Natural killer cell activation (Immune System Process)	4.06E-03
DNA-dependent transcription (Metabolic Process)	7.61E-03
Ion transport (Localization)	1.81E-02
Protein phosphorylation (Metabolic Process)	3.31E-02
Cellular component morphogenesis (Cellular Component Organization or Biogenesis)	3.85E-02
Cellular component organization (Cellular Component Organization or Biogenesis)	7.58E-04
Cell communication (Cellular Process)	2.57E-02
Biological regulation	3.03E-05
Biological adhesion	4.67E-02
GO-Molecular Function	
Transferase activity (Catalytic Activity)	1.67E-06
Hydrolase activity (Catalytic Activity)	2.87E-04
Kinase activity (Catalytic Activity)	7.73E-03
Protein binding (Binding)	2.99E-03
DNA binding (Binding)	4.19E-03
Transmembrane transporter activity (Transporter Activity)	3.37E-03
Sequence-specific DNA binding transcription factor activity (Nucleic Acid Binding Transcription Factor Activity)	1.52E-02
Structural constituent of ribosome (Structural Molecule Activity)	3.59E-02

Overrepresentation was determined by calculating the probability that the number of differentially expressed genes belonging to a particular category is larger or smaller than what would be expected based on a reference human genome. P-values are adjusted using a Bonferroni correction.

doi:10.1371/journal.pone.0145322.t004

Gene Set Enrichment Analysis

One limitation of a class or pathway overrepresentation analysis is that it does not indicate which condition is associated with the overrepresentation; GSEA does. Expressed genes were ranked by their correlation with the malignant phenotype and then this list was compared to sets of genes in a pathway, linking pathway enrichment to a phenotype. The more highly-correlated genes in a gene set, the higher the significance of that gene set. The gene sets with the highest normalized enrichment scores are presented in [Table 5](#) and other results are listed in the Supporting Information ([S7 Table](#)). The FDR cutoff was set at 25% to maximize hypothesis generation. Only one pathway was enriched in the tumor samples, the “RanMS pathway”

Table 5. Significant gene sets enriched in malignant and non-malignant prostate with the largest normalized enrichment scores.

Gene Set	ES	NES	P-value	Q-value
BioCarta: RanMS pathway	0.827	1.652	4.02E-03	2.05E-01
KEGG: Calcium signaling pathway	-0.456	-1.714	4.89E-03	5.75E-02
KEGG: Basal cell carcinoma	-0.482	-1.647	6.59E-03	6.10E-02
KEGG: Oxytocin signaling pathway	-0.443	-1.603	1.66E-02	6.26E-02
KEGG: Thyroid hormone synthesis	-0.466	-1.652	1.19E-02	6.34E-02
KEGG: Signaling pathways regulating pluripotency of stem cells	-0.432	-1.596	4.56E-03	6.35E-02
KEGG: Prolactin signaling pathway	-0.461	-1.605	4.52E-03	6.44E-02
KEGG: Pathways in cancer	-0.433	-1.624	8.77E-03	6.49E-02
KEGG: ECM-receptor interaction	-0.517	-1.633	3.76E-02	6.52E-02
KEGG: cAMP signaling pathway	-0.434	-1.719	0.00E+00	6.63E-02
KEGG: MAPK signaling pathway	-0.417	-1.614	2.28E-03	6.78E-02
KEGG: Regulation of actin cytoskeleton	-0.449	-1.606	8.99E-03	6.84E-02
KEGG: Phosphatidylinositol signaling pathway	-0.517	-1.653	4.38E-03	6.93E-02
KEGG: cGMP-PKG signaling pathway	-0.469	-1.677	1.43E-02	7.12E-02
KEGG: TGF-beta signaling pathway	-0.513	-1.654	1.11E-02	7.73E-02
KEGG: Focal adhesion	-0.517	-1.725	1.61E-02	7.87E-02
KEGG: Hippo signaling pathway	-0.482	-1.733	0.00E+00	9.65E-02
KEGG: Proteoglycans in cancer	-0.492	-1.740	0.00E+00	1.38E-01
KEGG: Ras signaling pathway	-0.460	-1.760	2.23E-03	2.13E-01
BioCarta: p38 MAPK pathway	-0.527	-1.584	8.15E-03	2.48E-01

ES = enrichment score, NES = normalized enrichment score, Q-value = false discovery rate-adjusted P-value. Positive enrichment scores correspond to enrichment in the malignant samples. Negative enrichment scores correspond to enrichment in the non-malignant samples.

doi:10.1371/journal.pone.0145322.t005

which includes the genes that regulate the formation of the mitotic spindle during cell division. Ten genes in our list of DEGs belonged to this pathway, each contributing to its enrichment in the malignant phenotype (Table 6). All ten were differentially expressed and up-regulated in

Table 6. Differentially Expressed Ran-Mitotic Spindle Pathway Components in Human Prostate Cancer.

Ran regulation of mitotic spindle formation pathway			
Gene Name	Symbol	Expression	Role
GTP-binding nuclear protein Ran	<i>RAN</i>	↑	GTPase; nuclear transport; formation of mitotic spindle [74]
Regulator of chromosome condensation	<i>RCC1</i>	↑	Guanine nucleotide exchange factor of Ran, produces a RanGTP gradient around chromosomes. [75]
Ran GTPase-activating protein 1	<i>RANGAP1</i>	↑	Accelerates RanGTP hydrolysis, helps maintain RanGTP gradient around chromosomes. [75]
Ran binding protein 1	<i>RANBP1</i>	↑	Regulates activity of RCC1 and RANGAP [76, 77]
Importin subunit alpha-1	<i>KPNA2</i>	↑	Nuclear import; KPNA2 adapter protein [78]
Importin subunit beta-1	<i>KPNB1</i>	↑	Nuclear import; docking platform [79, 80]
Targeting protein for Xklp2	<i>TPX2</i>	↑	Spindle assembly factor; microtubule nucleation, separation of bipolar mitotic spindle [81, 82]
Nuclear mitotic apparatus protein 1	<i>NUMA1</i>	↑	Spindle assembly factor; Establishes, maintains mitotic spindle poles. [83]
Kinesin-like protein KIF15	<i>KIF15</i>	↑	Spindle assembly factor; Bipolar spindle maintenance, elongation [82]
Aurora kinase A	<i>AURKA</i>	↑	Centrosome maturation, separation, and centrosomal microtubule stabilization and nucleation. [84]

↑ = up-regulated expression, ↓ = down-regulated expression

doi:10.1371/journal.pone.0145322.t006

Table 7. Significantly impacted pathways in human prostate cancer as determined by SPIA.

Name	NDE/pSize	pNDE	pPERT	pG	pGFdr
Proteoglycans in cancer	140/201	1.75E-05	5.00E-06	2.11E-09	1.86E-07
Hippo signaling pathway	112/153	3.12E-06	1.60E-02	8.90E-07	3.91E-05
Pathways in cancer	257/398	7.77E-05	2.00E-03	2.59E-06	7.60E-05
Focal adhesion	144/207	1.48E-05	2.10E-02	4.97E-06	1.09E-04
cGMP-PKG signaling pathway	118/167	2.80E-05	3.07E-01	1.09E-04	1.92E-03
Calcium signaling pathway	115/180	1.08E-02	4.00E-03	4.78E-04	7.01E-03
Ras signaling pathway	144/225	4.36E-03	2.90E-02	1.26E-03	1.59E-02
TGF-β signaling pathway	60/80	1.95E-04	8.54E-01	1.62E-03	1.78E-02
Chronic myeloid leukemia	55/73	2.93E-04	8.74E-01	2.37E-03	2.32E-02
Basal cell carcinoma	36/55	8.03E-02	6.00E-03	4.16E-03	3.59E-02
Regulation of actin cytoskeleton	135/213	9.11E-03	6.80E-02	5.20E-03	3.59E-02
ErbB signaling pathway	60/87	5.98E-03	1.07E-01	5.34E-03	3.59E-02
Glioma	48/65	1.50E-03	4.51E-01	5.60E-03	3.59E-02
Small cell lung cancer	58/86	1.38E-02	5.00E-02	5.71E-03	3.59E-02
Oxytocin signaling pathway	105/157	1.82E-03	4.53E-01	6.69E-03	3.87E-02
MAPK signaling pathway	152/254	7.51E-02	1.30E-02	7.75E-03	3.87E-02
Cell cycle	85/124	1.60E-03	6.41E-01	8.07E-03	3.87E-02
MicroRNAs in cancer	97/149	8.72E-03	NA	8.72E-03	3.87E-02
cAMP signaling pathway	132/200	1.15E-03	9.75E-01	8.76E-03	3.87E-02
Rap1 signaling pathway	130/211	3.42E-02	3.30E-02	8.80E-03	3.87E-02

NDE = number of differentially expressed elements, pSize = pathway size, pNDE = overrepresentation probability, pPERT = perturbation probability, pG = global probability, pGFdr = false discovery rate-adjusted global probability. Bold pathways were also significant by Gene Set Enrichment Analysis.

doi:10.1371/journal.pone.0145322.t007

the malignant samples. The remaining pathways were enriched in the non-malignant phenotype. The most significant pathway enriched in the non-malignant phenotype was the “calcium signaling” pathway. Enrichment of the calcium signaling pathway was due to 81 DEGs and 19 other genes or transcripts (S8 Table). Also enriched in the non-malignant phenotype were several other signaling pathways (oxytocin, prolactin, cAMP, MAPK, cGMP-PKG, TGF-β, Hippo, and Ras) and pathways related to cell-cell and cell-matrix adhesion (extracellular matrix-receptor interaction, actin cytoskeleton regulation, proteoglycans, and focal adhesion).

Signaling Pathway Impact Analysis

SPIA considers whether or not the DEGs found in a pathway have a meaningful impact within that pathway and thus addresses the topology of DEGs in pathways [66]. In other words, pathway significance is partly dependent on if the number of DEGs observed in a pathway is larger than that observed by random chance. This is captured in the probability of overrepresentation. Pathway significance is also partly based on whether DEGs in a particular pathway are at crucial junctions and can thus perturb the pathway. This is the probability of perturbation. These two probabilities are combined into a global probability which is adjusted by the false discovery rate. This adjusted metric was used to rank the impact of the pathways. Many of the same pathways were identified as significant in both GSEA and SPIA analysis (Table 7). In fact, the 8 most significant pathway results from SPIA were all significantly enriched in GSEA. However, only the “calcium signaling” pathway was highly ranked in both analyses. The only pathway activated in the malignant condition was the “TGF-β signaling” pathway (Table 8). The other pathways were all inhibited in the malignant condition. Similar to GSEA results,

Table 8. Components of the TGF- β Signaling Pathway Differentially Expressed in Human Prostate Cancer.

TGF- β Signaling Pathway			
Gene Name	Symbol	Expression	Role
Transforming growth factor β -2	<i>TGF-β2</i>	↓	Cytokine growth factor [85]
Transforming growth factor β -3	<i>TGF-β3</i>	↓	Cytokine growth factor [85]
TGF- β receptor type I	<i>TGFBR1</i>	↓	transmembrane serine/threonine kinase [86]
TGF- β receptor type II	<i>TGFBR2</i>	↓	transmembrane serine/threonine kinase [86]
TGF- β receptor type III	<i>TGFBR3</i>	↓	non-signaling receptor, presents TGF- β ligands to TGFBR2 [86]
Latent-transforming growth factor β -binding protein 1	<i>LTBP1</i>	↓	maintains latency of TGF- β [87]
Mothers against decapentaplegic homolog 2	<i>SMAD2</i>	↓	receptor SMAD for TGFBR1 [88]
Mothers against decapentaplegic homolog 3	<i>SMAD3</i>	↓	receptor SMAD for TGFBR1 [88]
Mothers against decapentaplegic homolog 4	<i>SMAD4</i>	↓	complexes with receptor SMADs before nuclear translocation [88]
Mothers against decapentaplegic homolog 7	<i>SMAD7</i>	↓	blocks phosphorylation of SMAD 2/3 [89]
E3 ubiquitin-protein ligase RBX1	<i>RBX1</i>	↑	In complex with CUL1 degrades SMAD2/3 [90]
Cullin-1	<i>CUL1</i>	↓	In complex with RBX1 degrades SMAD2/3 [90]
Retinoblastoma-like protein 1	<i>RBL1</i>	↓	E2F4/5 corepressor of <i>myc</i> [91]
Transcription factor E2F4	<i>E2F4</i>	↓	<i>myc</i> transcription factor [91]
Transcription factor E2F5	<i>E2F5</i>	↑	<i>myc</i> transcription factor [91]
Myc proto-oncogene protein	<i>MYC</i>	↑	Influences cell growth, cell cycle, apoptosis, metabolism, energy production, DNA replication and RNA stability and splicing [92]
Sp1 Transcription factor	<i>SP1</i>	↓	Transcription factor regulating growth factors, DNA synthesis regulators, and cell cycle genes including CDKN2B [93, 94]
Cyclin-dependent kinase 4 inhibitor B	<i>CDKN2B</i>	↓	Mediates cell cycle arrest at G1 [95]

↑ = up-regulated, ↓ = down-regulated

doi:10.1371/journal.pone.0145322.t008

several signaling pathways (oxytocin, cAMP, MAPK, cGMP-PKG, TGF- β , Hippo, Rap1, ErbB, and Ras) and pathways related to cell-cell and cell-matrix adhesion (proteoglycans, focal adhesion, and actin cytoskeleton regulation) were impacted. Images of the pathways with DEGs highlighted can be accessed in the Supporting Information (S9 Table).

Discussion

Global expression studies have documented many differentially expressed genes in human prostate cancer [7, 9, 13–15, 96–102]. Lucas and Heath compiled a list of differentially expressed genes with reported prognostic significance in prostate cancer [30]. Of the 22 genes listed, 19 were differentially expressed in our TCGA dataset and there was agreement in expression pattern between 12 genes. *PTEN*, *TMPRSS2*, *MYC*, *SMAD4*, *EZH2*, *p53*, *BCL2*, *NPY*, *PLA2G7*, *Ki-67*, *p16*, and *BAX* expression in our findings matched what was presented in the literature. *PTEN*, a tumor suppressor, was down-regulated in malignant samples. The deletion of *PTEN* correlates with higher Gleason grade, risk of progression, and recurrence after therapy, and advanced localized or metastatic disease and death [103, 104]. *SMAD4* was down-regulated in our TCGA prostate cancer data and has also been found to be down-regulated in prostate cancers, including advanced tumors [105, 106]. The deletion of this gene has led to invasive, metastatic, and lethal prostate cancers in a mouse model [39]. *TMPRSS2* was up-

regulated and this is in agreement with reports of it being more highly expressed in prostate carcinoma compared to normal prostate epithelium [107, 108]. *TMPRSS2* contributes to the invasion and metastasis of prostate cancer [109]. Further, *TMPRSS2-ERG* gene fusion holds promise as a potential prostate cancer biomarker [110]. *MYC* was also up-regulated in this dataset and the overexpression (gene amplification, mRNA, and protein increase) of *MYC* in prostate cancer is well-documented [111–115]. *MYC* gene amplification was found more often in metastases [116, 117] and also correlated with poor prognostic factors like higher Gleason and histopathological scores [118], or greater chance of PSA recurrence [114]. *EZH2* up-regulation is reported here and in the literature where such overexpression led to increased proliferation in prostate cells and is associated with aggressive disease and increased risk of recurrence [119]. The expression of *p53* mRNA was increased in malignant samples in our TCGA data. In a study of prostate cancer patients, *p53* positive expression was seen in the majority (69.1%) of patients with the number of positive patients increasing as stage and Gleason score increased. *P53* was also an independent predictor of recurrence [120]. *BCL2* mRNA expression was decreased in TCGA tumor samples. The absence of BCL-2 protein expression is reported in prostate cancer [120, 121]. Furthermore, BCL-2 expression is negative in androgen-dependent, but increased in hormone insensitive prostate cancers [122–124] and correlated with poor prognosis [125]. Pro-neuropeptide Y was up-regulated in this study and in the literature [126, 127]. Pro-neuropeptide Y up-regulation is associated with non-aggressive tumors [128] and regulates proliferation in prostate cancer cell lines [129]. *PLA2G7* was up-regulated in our data. It is reported to be more highly expressed in prostate cancer compared to benign samples [130, 131] and the TCGA samples studied here. Levels of *Ki-67* mRNA were increased in tumor versus non-malignant samples from our TCGA data and in the literature compared to normal tissue [132]. Furthermore *Ki-67* protein is increased in prostate cancer [133–136], prostate cancer metastases [137, 138] and is a useful prognostic marker [139]. In our list of DEGs, *p16* was up-regulated. Recently, *p16* expression was found in a large majority of prostate tissues [140]. *BAX* mRNA expression was increased in this TCGA dataset and *BAX* protein had increased expression in prostate cancer [141].

The remaining 7 DEGs in common with Lucas and Heath's list displayed a discrepancy in expression pattern between our results and the literature. *TGF- β 1* was not differentially expressed but *TGF- β 2* was down-regulated. Expression of *TGF- β 1* and *TGF- β 2* was increased in prostate cancer compared to normal or non-malignant tissues [142–147]. However, *TGF- β 3* was down-regulated in agreement with other reports of *TGF- β 3* expression in prostate cancer [97, 148]. Both α and β isoforms of *IL-1* and *IL-6* were down-regulated in this TCGA dataset. *IL-1 α* and *IL-6* were up-regulated in prostate cancer samples [149–153]. *IL-6* stimulated growth of LNCaP cells [154] and elevated *IL-6* was also associated with poor prognosis in prostate cancer [149, 155–162]. *IL-1 β* has been reported both up- and down-regulated in the literature. Protein expression in patient samples was down-regulated [163] but elevated gene and protein expression in human cancer cells and tumors has also been reported [164]. In our list of DEGs, *p21* was down-regulated. Aaltomaa *et al.* reported *p21* protein expression in the majority of prostate tumors but not in normal prostate epithelial cells [165] but other studies reported *p21* immunostaining in only 20%–35% of cancer samples [166, 167]. Both *p21* and *p16* inhibited growth in prostate cancer cell lines [168]. Vascular endothelial growth factor A (*VEGF-A*) was down-regulated in our data. High expression of *VEGF* correlated with poor prognosis [169], but some studies reported that the higher expression of *VEGF-A* correlated with better clinical outcome [170]. *TRAIL/TNFSF10* was up-regulated in our TCGA data. While epithelial expression of TRAIL protein was stronger in tumors, stromal expression of TRAIL was decreased or absent in tumors [171, 172]. Only stromal TRAIL expression correlated with recurrence-free survival [171]. *NFKB1* was down-regulated in our data. However,

NFKB1 protein expression progressively increased in normal, benign prostatic hyperplasia and prostate cancer tissues [173]. The other DEGs with prognostic significance in prostate cancer that were not differentially expressed in our list of DEGs include *IL-7*, *CCL-2*, and *CDH1*.

Comparisons between the DEGs presented herein and DEGs listed in other studies highlight variance from experiment to experiment. Despite such variance a strong underlying correlation between datasets may still sometimes be seen [174]. These correlations would most likely be captured in a pathway approach. Thus our TCGA data was subject to pathway analysis. We found the “Ran regulation of mitotic spindle formation” pathway to be most significant in prostate cancer and the “TGF- β signaling” pathway to be activated in prostate cancer. Additionally, the following pathways were significant across both GSEA and SPIA methods and were associated with the non-malignant samples and were inhibited in the tumor samples: “proteoglycans in cancer”, “Hippo signaling pathway”, “cGMP-PKG signaling pathway”, “Ras signaling pathway”, “MAPK signaling pathway”, “Focal adhesion”, “Regulation of actin cytoskeleton”, “Oxytocin signaling pathway”, and “Pathways in cancer”.

Ran regulation of mitotic spindle formation pathway

Ran is a small GTPase of the Ras family known to function in directing nucleocytoplasmic transport, in cell cycle control through regulation of transition to S-phase and mitosis, and in regulating the mitotic spindle during mitosis and the reassembly of the nuclear envelope after mitosis [175]. Ran’s control over the mitotic spindle is the pathway that was shown to be significant in prostate cancer in our data. Proper functioning of this pathway assembles spindle microtubules for chromosome alignment and segregation in a way that ensures a single copy of each chromosome is distributed to the daughter cells, thus avoiding aneuploidy [74, 75, 176]. Each of the genes in this pathway, which include Ran, its regulators, accessory proteins, spindle assembly factors, and import/export factors, was up-regulated (Table 6). Ran’s function in mitotic spindle assembly is reviewed by Clarke and Zhang [176]. Ran-GDP is converted to Ran-GTP by the guanine nucleotide-exchange factor RCC1 and is hydrolyzed back to Ran-GDP with the aid of the GTPase activating protein RanGAP1 and RanBP1/2. The specific localization of RCC1 and RanGAP1/RanBP2 results in the formation of Ran-GTP at precise points along spindle assembly. Importin- α /importin- β complexes carry spindle assembly factors such as TPX2 and NuMA into the nucleus where they are released at chromosomes after interaction with Ran-GTP. Spindle assembly factors then interact with other molecules such as Aurora kinase A to form the spindle.

Ran-GTP overexpression was reported in various human cancers [177–181] and multiple cancer cell lines [181, 182]. Ran proved critical for epithelial ovarian cancer cell survival [183] and its overexpression caused malignant transformation in rat mammary cells [184]. Silencing of Ran in tumor cell lines, but not normal cells, led to cell death after aberrations in mitotic spindle assembly and mitochondrial function [181]. In agreement with our data, other pathway components and Ran-associated genes are also overexpressed in cancer: Aurora kinase A [185], TPX2 [186–188], and HSET [189]. Ran has not been extensively studied in prostate cancer. There are reports of increased Ran expression in prostate tumor tissues [190] and Ran functions as an androgen receptor coactivator [191, 192].

TGF- β Signaling

The TGF- β signaling pathway was activated in the malignant condition in this TCGA prostate cancer dataset. In general, TGF- β signaling regulates cell proliferation, migration, differentiation, epithelial-mesenchymal transition, immune-suppression, and apoptosis [85, 193]. Several components of the TGF- β signaling pathway were differentially expressed (Table 8). The

binding of active TGF- β to its receptors begins a phosphorylation cascade that activates Smad transcription factors which translocate to the nucleus. In the nucleus, the Smad complex binds various transcription factors, coactivators, co-repressors, and chromatin remodeling factors to regulate gene expression [194, 195]. Ultimately, TGF- β signaling promotes expression of inhibitors of cell cycle progression and suppresses proliferative genes [195, 196]. Tumor cells can subvert the suppressive effect of TGF- β signaling seen in normal cells to promote tumorigenesis [194, 195].

Several studies have reported the increase of TGF- β isoforms in prostate cancer [142, 145–147, 197, 198], however our study shows a significant decrease in *TGF- β 2* and *TGF- β 3* gene expression and no differential expression of *TGF- β 1*. Our results are, however, corroborated by the work of Dallas *et al.* which showed both latent and active forms of TGF- β 2 were decreased in malignant prostate cells compared to normal prostate epithelial cells cultured from the same patient [199]. Our results are also corroborated by studies showing lost or decreased expression of TGF- β 3 [143, 148]. In our TCGA dataset, all three TGF- β receptors were down-regulated. Loss of TGF- β receptors is consistent with literature [146, 200–204] and represents a mechanism through which tumors avoid growth suppression by TGF- β , thus facilitating the development of cancer after oncogenic triggers [195]. Additionally, down-regulation of TGF- β 1, β 2, and β 3 is associated with androgen-stimulated growth of prostate cancer cells [205].

Although TGF- β signaling typically operates through Smad proteins, the pathway signal may also be diverted through other Smad-independent pathways like PI3K/AKT, ERK/MAPK, JNK/p38 MAPK and Rho-like GTPase signaling pathways [151, 206]. Since Smad genes were down-regulated, we looked at other effectors and found serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha and beta isoforms (*PPP2R1A*, *PPP2R1B*) to be up-regulated along with the targets ribosomal protein S6 kinase β -1 and β -2 (*RPS6KB1*, *RPS6KB2*), the serine/threonine-protein phosphatase 2A catalytic subunit β isoform (*PPP2CB*) was down-regulated, both *RhoA* and *ROCK1* were down-regulated and *MAPK1* and *MAPK3* were also down-regulated. In our TCGA data, MAPK signaling pathway was significantly different between tumor and non-malignant samples, however it was more associated with non-malignant samples whereas TGF- β was more associated with tumor samples. Erk signaling alters the expression of genes controlling cell motility, and cell-matrix adhesion and interactions [207]. Cell motility and cell-matrix adhesion-related gene sets were also significantly enriched in the non-malignant samples of our TCGA prostate cancer data (Table 5).

Pathway Comparison

There were a few surprising results from GSEA analysis—namely, the significance of prolactin and oxytocin signaling pathways and thyroid hormone synthesis pathway. The genes contributing to the enrichment of these pathways in non-malignant samples were not the namesake hormones themselves, but the multiple kinases, phosphatases, and calcium or potassium channel proteins that participate in hormone signaling (S10–S12 Tables). In the case of oxytocin signaling, the pathway operates through both calcium signaling and MAPK signaling (S1 Fig), which were also found to be significant. For the prolactin pathway (S2 Fig), the enrichment of MAPK kinases and PI3K kinases is abundant however prolactin itself is not enriched (S11 Table). Finally, for thyroid synthesis pathway (S3 Fig), none of the hormones or receptors are present but other components through which they operate are (S12 Table). Thus it appears these pathways could have been flagged due to substantial overlap with the signaling of other pathways since neither oxytocin, prolactin, or thyroid stimulating hormone nor their receptors were differentially expressed. These results demonstrate the limitation of GSEA discussed previously, the topology of genes in the pathways is unaccounted for. SPIA is a complementary

pathway method that does consider the position of genes in the pathway. It is noteworthy that SPIA analysis was able to filter prolactin and thyroid hormone synthesis pathways from significant results.

Comparison to previous pathway studies which used microarray data or single nucleotide polymorphisms from genome-wide association studies showed that several pathways were identified across experimental platforms. Savli *et al.* looked at gene networks and pathway analysis in prostate cancer [208]. However, that study used microarray to measure gene expression and found 738 up-regulated genes and 515 down-regulated genes. This study used RNA sequencing data and found 5,736 up-regulated genes and 5,379 down-regulated genes. Some advantages of a sequencing method over microarray approach include more extensive transcript identification beyond the coverage of sequence libraries although correlation between some sequencing approaches and microarray platforms has been demonstrated [34]. Additionally, our patient pool was much larger (173 versus 21 tumor and 52 versus 10 non-malignant). The methods for identifying pathways was also different. Savli *et al.* used Ingenuity Pathway Analysis to identify pathways and construct gene networks. “Axonal guidance signaling” (down-regulated) and “acute phase response” (up-regulated) were the most significant pathways among the up- and down-regulated canonical pathways reported by Savli *et al.* but were not found in this study’s results. However other important pathways in prostate cancer were found in both studies including “actin cytoskeleton” (down-regulated in both), “calcium signaling” (up-regulated in Savli *et al.*, down-regulated in ours), and “MAPK signaling” (down-regulated in both). Jia *et al.* used a combination of GSEA and Plink set-based tests on microarray data and genome-wide association studies to identify thirteen KEGG pathways involved in prostate cancer [209]. In this study, we found five of these KEGG pathways to be important in prostate cancer: regulation of actin cytoskeleton, small cell lung cancer, cell cycle, chronic myeloid leukemia, and TGF- β signaling pathway.

Conclusion

This work presents a comprehensive gene expression profile of human prostate cancer. Differential gene expression was analyzed in the context of gene sets and pathways to identify signature pathways associated with prostate cancer. “TGF- β signaling” and “Ran regulation of mitotic spindle formation” pathways were strongly associated with prostate cancer. Since it is an underexplored area in prostate cancer, we suggest Ran pathway components for further investigation in prostate cancer pathogenesis. Several other significant pathways confirm reported findings from microarray data that suggest actin cytoskeleton regulation, cell cycle, MAPK signaling, and calcium signaling are also altered in prostate cancer. We further observed that none of the most highly altered genes with the largest increases or decreases in expression appeared in the significant pathways. Thus we have demonstrated that both differential expression and pathway analysis are important in extracting meaningful information.

Supporting Information

S1 Fig. KEGG Oxytocin Signaling Pathway. Differentially expressed genes are highlighted in red.

(PNG)

S2 Fig. KEGG Prolactin Signaling Pathway. Differentially expressed genes are highlighted in red.

(PNG)

S3 Fig. KEGG Thyroid Hormone Synthesis Pathway. Differentially expressed genes are highlighted in red.

(PNG)

S1 Table. Age- and stage matched human prostate cancer mRNA expression dataset. This file includes the RNAseq expression data for the 225 age- and stage-matched prostate cancer non-malignant and tumor samples downloaded from The Cancer Genome Atlas and used for the analyses presented in this work.

(XLSX)

S2 Table. Selected KEGG pathways used for all pathway analyses. This is the set of KEGG pathways used in Gene Set Enrichment Analysis and Signaling Pathway Impact Analysis. Pathways likely to have little relevance to prostate cancer (*e.g.* parasitic, bacterial, and viral infectious diseases, substance dependencies, and specific immune, neurodegenerative, and cardiovascular diseases) have been excluded.

(XLSX)

S3 Table. Differentially expressed genes in human prostate cancer. A total of 11,115 genes and transcripts were differentially expressed according to DESeq2 analysis using Wald statistics. All statistical parameters plus the calculated \log_2 fold change are presented.

(XLSX)

S4 Table. Complete DESeq2 analysis results including all genes and transcripts evaluated. Complete results of DESeq2 analysis with statistical parameters and calculated \log_2 fold change.

(XLSX)

S5 Table. Classification of differentially expressed genes by protein class and gene ontology. Complete classification based on PANTHER protein class, GO Molecular Function and GO Biological Process terms.

(XLSX)

S6 Table. PANTHER overrepresentation results for protein class and gene ontology. This is the full pathway overrepresentation analysis of protein class and GO Biological Process categories among DEGs from the dataset.

(XLSX)

S7 Table. Complete GSEA results for BioCarta, Reactome and KEGG gene sets.

(XLSX)

S8 Table. Genes and Transcripts contributing to KEGG Calcium Signaling Pathway enrichment in GSEA. These genes and transcripts from the evaluated TCGA dataset contribute to the enrichment of the KEGG Calcium Signaling Pathway in non-malignant samples.

(XLSX)

S9 Table. Complete SPIA results for KEGG pathways.

(XLSX)

S10 Table. Genes contributing to KEGG Oxytocin Signaling Pathway enrichment. These genes and transcripts from the evaluated TCGA dataset contribute to the enrichment of the KEGG Calcium Signaling Pathway in non-malignant samples.

(XLSX)

S11 Table. Genes contributing to KEGG Prolactin Signaling Pathway enrichment. These genes and transcripts from the evaluated TCGA dataset contribute to the enrichment of the KEGG Calcium Signaling Pathway in non-malignant samples.

(XLSX)

S12 Table. Genes contributing to KEGG Thyroid Hormone Synthesis Pathway enrichment. These genes and transcripts from the evaluated TCGA dataset contribute to the enrichment of the KEGG Calcium Signaling Pathway in non-malignant samples.

(XLSX)

Acknowledgments

The authors thank Dr. Jinfeng Zhang and Mr. Kaixian Yu in the Departments of Statistics at Florida State University for helpful discussions. This work was in part supported by the Leslie N. Wilson-Delores Auzenne Graduate Assistantship for Minorities awarded to JSM by the Florida State University Graduate School, the Research Experience Program of Women in Math, Science, and Engineering of Florida State University to AKVL, and grants from the Florida State University and an Endowed Chair Professorship in Cancer Research from anonymous donors to QXAS. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceived and designed the experiments: QXAS JSM. Performed the experiments: JSM AKVL CJR. Analyzed the data: QXAS JSM AKVL CJR. Contributed reagents/materials/analysis tools: QXAS JSM AKVL CJR. Wrote the paper: QXAS JSM AKVL CJR.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. 2015; 65(1): 5–29.
2. Draisma G, Etzioni R, Tsodikov A, Mariotto A, Wever E, Gulati R, et al. Lead time and overdiagnosis in prostate-specific antigen screening: importance of methods and context. *J Natl Cancer Inst.* 2009; 101(6): 374–383. doi: [10.1093/jnci/djp001](https://doi.org/10.1093/jnci/djp001) PMID: [19276453](https://pubmed.ncbi.nlm.nih.gov/19276453/)
3. Hoffman RM. Screening for Prostate Cancer. *N Engl J Med.* 2011; 365(21): 2013–2019. doi: [10.1056/NEJMcp1103642](https://doi.org/10.1056/NEJMcp1103642) PMID: [22029754](https://pubmed.ncbi.nlm.nih.gov/22029754/)
4. Moyer VA, U.S. Preventive Services Task Force. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med.* 2012; 157(2): 120–134. doi: [10.7326/0003-4819-157-2-201207170-00459](https://doi.org/10.7326/0003-4819-157-2-201207170-00459) PMID: [22801674](https://pubmed.ncbi.nlm.nih.gov/22801674/)
5. Cazares LH, Adam BL, Ward MD, Nasim S, Schellhammer PF, Semmes OJ, et al. Normal, benign, preneoplastic, and malignant prostate cells have distinct protein expression profiles resolved by surface enhanced laser desorption/ionization mass spectrometry. *Clin Cancer Res.* 2002; 8(8): 2541–2552. PMID: [12171882](https://pubmed.ncbi.nlm.nih.gov/12171882/)
6. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, et al. Delineation of prognostic biomarkers in prostate cancer. *Nature.* 2001; 412(6849): 822–826. doi: [10.1038/35090585](https://doi.org/10.1038/35090585) PMID: [11518967](https://pubmed.ncbi.nlm.nih.gov/11518967/)
7. Ernst T, Hergenbahn M, Kenzelmann M, Cohen CD, Bonrouhi M, Weninger A, et al. Decrease and gain of gene expression are equally discriminatory markers for prostate carcinoma: a gene expression analysis on total and microdissected prostate tissue. *Am J Pathol.* 2002; 160(6): 2169–2180. PMID: [12057920](https://pubmed.ncbi.nlm.nih.gov/12057920/)
8. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proc Natl Acad Sci U S A.* 2004; 101(3): 811–816. doi: [10.1073/pnas.0304146101](https://doi.org/10.1073/pnas.0304146101) PMID: [14711987](https://pubmed.ncbi.nlm.nih.gov/14711987/)
9. Luo J, Duggan DJ, Chen Y, Sauvageot J, Ewing CM, Bittner ML, et al. Human prostate cancer and benign prostatic hyperplasia: molecular dissection by gene expression profiling. *Cancer Res.* 2001; 61(12): 4683–4688. PMID: [11406537](https://pubmed.ncbi.nlm.nih.gov/11406537/)

10. Qian DZ, Huang CY, O'Brien CA, Coleman IM, Garzotto M, True LD, et al. Prostate cancer-associated gene expression alterations determined from needle biopsies. *Clin Cancer Res*. 2009; 15(9): 3135–3142. doi: [10.1158/1078-0432.CCR-08-1982](https://doi.org/10.1158/1078-0432.CCR-08-1982) PMID: [19366833](https://pubmed.ncbi.nlm.nih.gov/19366833/)
11. Stamey TA, Warrington JA, Caldwell MC, Chen Z, Fan Z, Mahadevappa M, et al. Molecular genetic profiling of Gleason grade 4/5 prostate cancers compared to benign prostatic hyperplasia. *J Urol*. 2001; 166(6): 2171–2177. PMID: [11696729](https://pubmed.ncbi.nlm.nih.gov/11696729/)
12. Yu YP, Landsittel D, Jing L, Nelson J, Ren B, Liu L, et al. Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. *J Clin Oncol*. 2004; 22(14): 2790–2799. doi: [10.1200/JCO.2004.05.158](https://doi.org/10.1200/JCO.2004.05.158)
13. Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, et al. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res*. 2001; 61(16): 5974–5978. PMID: [11507037](https://pubmed.ncbi.nlm.nih.gov/11507037/)
14. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, et al. Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell*. 2002; 1(2): 203–209. PMID: [12086878](https://pubmed.ncbi.nlm.nih.gov/12086878/)
15. LaTulippe E, Satagopan J, Smith A, Scher H, Scardino P, Reuter V, et al. Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res*. 2002; 62(15): 4499–4506. PMID: [12154061](https://pubmed.ncbi.nlm.nih.gov/12154061/)
16. Khan AP, Poisson LM, Bhat VB, Fermin D, Zhao R, Kalyana-Sundaram S, et al. Quantitative proteomic profiling of prostate cancer reveals a role for miR-128 in prostate cancer. *Mol Cell Proteomics*. 2010; 9(2): 298–312. doi: [10.1074/mcp.M900159-MCP200](https://doi.org/10.1074/mcp.M900159-MCP200) PMID: [19955085](https://pubmed.ncbi.nlm.nih.gov/19955085/)
17. Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell*. 2005; 8(5): 393–406. PMID: [16286247](https://pubmed.ncbi.nlm.nih.gov/16286247/)
18. Lin J, Xu J, Tian H, Gao X, Chen Q, Gu Q, et al. Identification of candidate prostate cancer biomarkers in prostate needle biopsy specimens using proteomic analysis. 2007; 121(12): 2596–2605.
19. Alaiya AA, Al-Mohanna M, Aslam M, Shinwari Z, Al-Mansouri L, Al-Rodayan M, et al. Proteomics-based signature for human benign prostate hyperplasia and prostate adenocarcinoma. *Int J Oncol*. 2011; 38(4): 1047–1057. doi: [10.3892/ijo.2011.937](https://doi.org/10.3892/ijo.2011.937) PMID: [21305254](https://pubmed.ncbi.nlm.nih.gov/21305254/)
20. Ahram M, Best CJM, Flaig MJ, Gillespie JW, Leiva IM, Chuaqui RF, et al. Proteomic analysis of human prostate cancer. *Mol Carcinog*. 2002; 33(1): 9–15. doi: [10.1002/mc.10019](https://doi.org/10.1002/mc.10019) PMID: [11807953](https://pubmed.ncbi.nlm.nih.gov/11807953/)
21. Meehan KL, Holland JW, Dawkins HJS. Proteomic analysis of normal and malignant prostate tissue to identify novel proteins lost in cancer. *Prostate*. 2002; 50(1): 54–63. doi: [10.1002/pros.10032](https://doi.org/10.1002/pros.10032) PMID: [11757036](https://pubmed.ncbi.nlm.nih.gov/11757036/)
22. Kiprijanovska S, Stavridis S, Stankov O, Komina S, Petrussevska G, Polenakovic M, et al. Mapping and Identification of the Urine Proteome of Prostate Cancer Patients by 2D PAGE/MS. *Int J Proteomics*. 2014; 2014: 594761. doi: [10.1155/2014/594761](https://doi.org/10.1155/2014/594761) PMID: [25215235](https://pubmed.ncbi.nlm.nih.gov/25215235/)
23. Davaliev K, Kostovska IM, Kiprijanovska S, Markoska K, Kubelka-Sabit K, Filipovski V, et al. Proteomics analysis of malignant and benign prostate tissue by 2D DIGE/MS reveals new insights into proteins involved in prostate cancer. *Prostate*. 2015; 75(14): 1586–1600. doi: [10.1002/pros.23034](https://doi.org/10.1002/pros.23034) PMID: [26074449](https://pubmed.ncbi.nlm.nih.gov/26074449/)
24. Glinsky GV, Glinskii AB, Stephenson AJ, Hoffman RM, Gerald WL. Gene expression profiling predicts clinical outcome of prostate cancer. *J Clin Invest*. 2004; 113(6): 913–923. doi: [10.1172/JCI20032](https://doi.org/10.1172/JCI20032) PMID: [15067324](https://pubmed.ncbi.nlm.nih.gov/15067324/)
25. Song C, Chen H, Wang T, Zhang W, Ru G, Lang J. Expression profile analysis of microRNAs in prostate cancer by next-generation sequencing. *Prostate*. 2015; 75(5): 500–516. doi: [10.1002/pros.22936](https://doi.org/10.1002/pros.22936) PMID: [25597612](https://pubmed.ncbi.nlm.nih.gov/25597612/)
26. Gordanpour A, Nam RK, Sugar L, Seth A. MicroRNAs in prostate cancer: from biomarkers to molecularly-based therapeutics. *Prostate Cancer Prostatic Dis*. 2012; 15(4): 314–319. doi: [10.1038/pcan.2012.3](https://doi.org/10.1038/pcan.2012.3) PMID: [22333688](https://pubmed.ncbi.nlm.nih.gov/22333688/)
27. Mahapatra S, Klee EW, Young CY, Sun Z, Jimenez RE, Klee GG, et al. Global methylation profiling for risk prediction of prostate cancer. *Clin Cancer Res*. 2012; 18(10): 2882–2895. doi: [10.1158/1078-0432.CCR-11-2090](https://doi.org/10.1158/1078-0432.CCR-11-2090) PMID: [22589488](https://pubmed.ncbi.nlm.nih.gov/22589488/)
28. Hoque MO, Topaloglu O, Begum S, Henrique R, Rosenbaum E, Van Criekinge W, et al. Quantitative methylation-specific polymerase chain reaction gene patterns in urine sediment distinguish prostate cancer patients from control subjects. *J Clin Oncol*. 2005; 23(27): 6569–6575. PMID: [16170165](https://pubmed.ncbi.nlm.nih.gov/16170165/)
29. Ozen M, Creighton CJ, Ozdemir M, Iltmann M. Widespread deregulation of microRNA expression in human prostate cancer. *Oncogene*. 2008; 27(12): 1788–1793. PMID: [17891175](https://pubmed.ncbi.nlm.nih.gov/17891175/)

30. Lucas SM, Heath EI. Current challenges in development of differentially expressed and prognostic prostate cancer biomarkers. *Prostate Cancer*. 2012; 2012: 640968. doi: [10.1155/2012/640968](https://doi.org/10.1155/2012/640968) PMID: [22970379](https://pubmed.ncbi.nlm.nih.gov/22970379/)
31. Coppola V, De Maria R, Bonci D. MicroRNAs and prostate cancer. 2010; 17(1): F1-F17.
32. Arora R, Koch MO, Eble JN, Ulbright TM, Li L, Cheng L. Heterogeneity of Gleason grade in multifocal adenocarcinoma of the prostate. *Cancer*. 2004; 100(11): 2362–2366. doi: [10.1002/cncr.20243](https://doi.org/10.1002/cncr.20243) PMID: [15160339](https://pubmed.ncbi.nlm.nih.gov/15160339/)
33. Ein-Dor L, Kela I, Getz G, Givol D, Domany E. Outcome signature genes in breast cancer: is there a unique set? *Bioinformatics*. 2005; 21(2): 171–178. doi: [10.1093/bioinformatics/bth469](https://doi.org/10.1093/bioinformatics/bth469) PMID: [15308542](https://pubmed.ncbi.nlm.nih.gov/15308542/)
34. Liu F, Jenssen T, Trimarchi J, Punzo C, Cepko CL, Ohno-Machado L, et al. Comparison of hybridization-based and sequencing-based gene expression technologies on biological replicates. *BMC Genomics*. 2007; 8(1): 153. doi: [10.1186/1471-2164-8-153](https://doi.org/10.1186/1471-2164-8-153)
35. Diamandis EP. The failure of protein cancer biomarkers to reach the clinic: why, and what can be done to address the problem? *BMC Med*. 2012; 10: 87-7015-10-87. doi: [10.1186/1741-7015-10-87](https://doi.org/10.1186/1741-7015-10-87)
36. Diamandis EP. Present and future of cancer biomarkers. *Clin Chem Lab Med*. 2014; 52(6): 791–794. doi: [10.1515/cclm-2014-0317](https://doi.org/10.1515/cclm-2014-0317) PMID: [24803613](https://pubmed.ncbi.nlm.nih.gov/24803613/)
37. Schrotten C, Dits NF, Steyerberg EW, Kranse R, van Leenders AGJLH, Bangma CH, et al. The additional value of TGFβ1 and IL-7 to predict the course of prostate cancer progression. 2011; 61(6): 905–910.
38. Latil A, Bieche I, Chene L, Laurendeau I, Berthon P, Cussenot O, et al. Gene expression profiling in clinically localized prostate cancer: a four-gene expression model predicts clinical behavior. *Clin Cancer Res*. 2003; 9(15): 5477–5485. PMID: [14654526](https://pubmed.ncbi.nlm.nih.gov/14654526/)
39. Ding Z, Wu C, Chu GC, Xiao Y, Ho D, Zhang J, et al. SMAD4-dependent barrier constrains prostate cancer growth and metastatic progression. *Nature*. 2011; 470(7333): 269–273. doi: [10.1038/nature09677](https://doi.org/10.1038/nature09677) PMID: [21289624](https://pubmed.ncbi.nlm.nih.gov/21289624/)
40. Bibikova M, Chudin E, Arsanjani A, Zhou L, Garcia EW, Modder J, et al. Expression signatures that correlated with Gleason score and relapse in prostate cancer. *Genomics*. 2007; 89(6): 666–672. doi: [10.1016/j.ygeno.2007.02.005](https://doi.org/10.1016/j.ygeno.2007.02.005) PMID: [17459658](https://pubmed.ncbi.nlm.nih.gov/17459658/)
41. Blume-Jensen P, Berman DM, Rimm DL, Shipitsin M, Putzi M, Nifong TP, et al. Development and clinical validation of an in situ biopsy-based multimer assay for risk stratification in prostate cancer. *Clin Cancer Res*. 2015; 21(11): 2591–2600. doi: [10.1158/1078-0432.CCR-14-2603](https://doi.org/10.1158/1078-0432.CCR-14-2603) PMID: [25733599](https://pubmed.ncbi.nlm.nih.gov/25733599/)
42. Knezevic D, Goddard AD, Natraj N, Cherbavaz DB, Clark-Langone KM, Snable J, et al. Analytical validation of the Oncotype DX prostate cancer assay—a clinical RT-PCR assay optimized for prostate needle biopsies. *BMC Genomics*. 2013; 14: 690. doi: [10.1186/1471-2164-14-690](https://doi.org/10.1186/1471-2164-14-690) PMID: [24103217](https://pubmed.ncbi.nlm.nih.gov/24103217/)
43. Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM, Reid JE, et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol*. 2011; 12(3): 245–255. doi: [10.1016/S1470-2045\(10\)70295-3](https://doi.org/10.1016/S1470-2045(10)70295-3) PMID: [21310658](https://pubmed.ncbi.nlm.nih.gov/21310658/)
44. Karnes RJ, Bergstralh EJ, Davicioni E, Ghadessi M, Buerki C, Mitra AP, et al. Validation of a genomic classifier that predicts metastasis following radical prostatectomy in an at risk patient population. *J Urol*. 2013; 190(6): 2047–2053. doi: [10.1016/j.juro.2013.06.017](https://doi.org/10.1016/j.juro.2013.06.017) PMID: [23770138](https://pubmed.ncbi.nlm.nih.gov/23770138/)
45. Zhang M, Yao C, Guo Z, Zou J, Zhang L, Xiao H, et al. Apparently low reproducibility of true differential expression discoveries in microarray studies. *Bioinformatics*. 2008; 24(18): 2057–2063. doi: [10.1093/bioinformatics/btn365](https://doi.org/10.1093/bioinformatics/btn365) PMID: [18632747](https://pubmed.ncbi.nlm.nih.gov/18632747/)
46. Chen J, Wang Y, Shen B, Zhang D. Molecular signature of cancer at gene level or pathway level? Case studies of colorectal cancer and prostate cancer microarray data. *Comput Math Methods Med*. 2013; 2013: 909525. doi: [10.1155/2013/909525](https://doi.org/10.1155/2013/909525) PMID: [23401724](https://pubmed.ncbi.nlm.nih.gov/23401724/)
47. Wang Y, Chen J, Li Q, Wang H, Liu G, Jing Q, et al. Identifying novel prostate cancer associated pathways based on integrative microarray data analysis. *Comput Biol Chem*. 2011; 35(3): 151–158. doi: [10.1016/j.compbiolchem.2011.04.003](https://doi.org/10.1016/j.compbiolchem.2011.04.003) PMID: [21704261](https://pubmed.ncbi.nlm.nih.gov/21704261/)
48. Abraham G, Kowalczyk A, Loi S, Haviv I, Zobel J. Prediction of breast cancer prognosis using gene set statistics provides signature stability and biological context. *BMC Bioinformatics*. 2010; 11(1): 277. doi: [10.1186/1471-2105-11-277](https://doi.org/10.1186/1471-2105-11-277)
49. Tian L, Greenberg SA, Kong SW, Altschuler J, Kohane IS, Park PJ. Discovering statistically significant pathways in expression profiling studies. *Proc Natl Acad Sci U S A*. 2005; 102(38): 13544–13549. PMID: [16174746](https://pubmed.ncbi.nlm.nih.gov/16174746/)

50. Lee E, Chuang HY, Kim JW, Ideker T, Lee D. Inferring pathway activity toward precise disease classification. *PLoS Comput Biol*. 2008; 4(11): e1000217. doi: [10.1371/journal.pcbi.1000217](https://doi.org/10.1371/journal.pcbi.1000217) PMID: [18989396](https://pubmed.ncbi.nlm.nih.gov/18989396/)
51. Chuang H, Lee E, Liu Y, Lee D, Ideker T. Network-based classification of breast cancer metastasis. *Mol Syst Biol*. 2007; 3: 140. doi: [10.1038/msb4100180](https://doi.org/10.1038/msb4100180) PMID: [17940530](https://pubmed.ncbi.nlm.nih.gov/17940530/)
52. Basu A, Drame A, Munoz R, Gijsbers R, Debysers Z, De Leon M, et al. Pathway specific gene expression profiling reveals oxidative stress genes potentially regulated by transcription co-activator LEDGF/p75 in prostate cancer cells. *Prostate*. 2012; 72(6): 597–611. doi: [10.1002/pros.21463](https://doi.org/10.1002/pros.21463) PMID: [21796653](https://pubmed.ncbi.nlm.nih.gov/21796653/)
53. Bettuzzi S, Davalli P, Astancolle S, Carani C, Madeo B, Tampieri A, et al. Tumor progression is accompanied by significant changes in the levels of expression of polyamine metabolism regulatory genes and clusterin (sulfated glycoprotein 2) in human prostate cancer specimens. *Cancer Res*. 2000; 60(1): 28–34. PMID: [10646846](https://pubmed.ncbi.nlm.nih.gov/10646846/)
54. Bettuzzi S, Scaltriti M, Caporali A, Brausi M, D'Arca D, Astancolle S, et al. Successful prediction of prostate cancer recurrence by gene profiling in combination with clinical data: a 5-year follow-up study. *Cancer Res*. 2003; 63(13): 3469–3472. PMID: [12839927](https://pubmed.ncbi.nlm.nih.gov/12839927/)
55. Mukherjee R, McGuinness DH, McCall P, Underwood MA, Seywright M, Orange C, et al. Upregulation of MAPK pathway is associated with survival in castrate-resistant prostate cancer. *Br J Cancer*. 2011; 104(12): 1920–1928. doi: [10.1038/bjc.2011.163](https://doi.org/10.1038/bjc.2011.163) PMID: [21559022](https://pubmed.ncbi.nlm.nih.gov/21559022/)
56. Waalkes S, Simon P, Hennenlotter J, Knapp J, Tezval H, Serth J, et al. Altered expression of Akt signaling pathway parameters in prostate needle biopsies derived from benign, adjacent and cancerous tissue. *Oncol Rep*. 2010; 23(5): 1257–1260. PMID: [20372838](https://pubmed.ncbi.nlm.nih.gov/20372838/)
57. Campa D, Husing A, Stein A, Dostal L, Boeing H, Pischon T, et al. Genetic variability of the mTOR pathway and prostate cancer risk in the European Prospective Investigation on Cancer (EPIC). *PLoS One*. 2011; 6(2): e16914. doi: [10.1371/journal.pone.0016914](https://doi.org/10.1371/journal.pone.0016914) PMID: [21373201](https://pubmed.ncbi.nlm.nih.gov/21373201/)
58. Kremer CL, Klein RR, Mendelson J, Browne W, Samadzede LK, Vanpatten K, et al. Expression of mTOR signaling pathway markers in prostate cancer progression. *Prostate*. 2006; 66(11): 1203–1212. doi: [10.1002/pros.20410](https://doi.org/10.1002/pros.20410) PMID: [16652388](https://pubmed.ncbi.nlm.nih.gov/16652388/)
59. Xu J, Lowey J, Wiklund F, Sun J, Lindmark F, Hsu FC, et al. The interaction of four genes in the inflammation pathway significantly predicts prostate cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2005; 14(11 Pt 1): 2563–2568. PMID: [16284379](https://pubmed.ncbi.nlm.nih.gov/16284379/)
60. Creighton CJ. Multiple oncogenic pathway signatures show coordinate expression patterns in human prostate tumors. *PLoS One*. 2008; 3(3): e1816. doi: [10.1371/journal.pone.0001816](https://doi.org/10.1371/journal.pone.0001816) PMID: [18350153](https://pubmed.ncbi.nlm.nih.gov/18350153/)
61. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.
62. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014; 15(12): 550–558. doi: [10.1186/s13059-014-0550-8](https://doi.org/10.1186/s13059-014-0550-8) PMID: [25516281](https://pubmed.ncbi.nlm.nih.gov/25516281/)
63. Mi H, Muruganujan A, Thomas PD. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. *Nucleic Acids Research*. 2013; 41(D1): 08/05/2015-D377-D386. doi: [10.1093/nar/gks1118](https://doi.org/10.1093/nar/gks1118)
64. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *PNAS*. 2005; 102(43): 15545–15550. doi: [10.1073/pnas.0506580102](https://doi.org/10.1073/pnas.0506580102) PMID: [16199517](https://pubmed.ncbi.nlm.nih.gov/16199517/)
65. Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M. KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res*. 2010; 38(Database issue): D355–60. doi: [10.1093/nar/gkp896](https://doi.org/10.1093/nar/gkp896) PMID: [19880382](https://pubmed.ncbi.nlm.nih.gov/19880382/)
66. Tarca AL, Draghici S, Khatri P, Hassan SS, Mittal P, Kim JS, et al. A novel signaling pathway impact analysis. *Bioinformatics*. 2009; 25(1): 75–82. doi: [10.1093/bioinformatics/btn577](https://doi.org/10.1093/bioinformatics/btn577) PMID: [18990722](https://pubmed.ncbi.nlm.nih.gov/18990722/)
67. Tarca AL, Kathri P and Draghici S. SPIA: Signaling Pathway Impact Analysis (SPIA) using combined evidence of pathway over-representation and unusual signaling perturbations. 2013.
68. Furuta E, Okuda H, Kobayashi A, Watabe K. Metabolic genes in cancer: their roles in tumor progression and clinical implications. *Biochim Biophys Acta*. 2010; 1805(2): 141–152. doi: [10.1016/j.bbcan.2010.01.005](https://doi.org/10.1016/j.bbcan.2010.01.005) PMID: [20122995](https://pubmed.ncbi.nlm.nih.gov/20122995/)
69. Robey RB, Weisz J, Kuemmerle NB, Salzberg AC, Berg A, Brown DG, et al. Metabolic reprogramming and dysregulated metabolism: cause, consequence and/or enabler of environmental carcinogenesis? *Carcinogenesis*. 2015; 36(Suppl 1): S203–S231. doi: [10.1093/carcin/bgv037](https://doi.org/10.1093/carcin/bgv037) PMID: [26106140](https://pubmed.ncbi.nlm.nih.gov/26106140/)
70. Dang CV. Links between metabolism and cancer. *Genes Dev*. 2012; 26(9): 877–890. doi: [10.1101/gad.189365.112](https://doi.org/10.1101/gad.189365.112) PMID: [22549953](https://pubmed.ncbi.nlm.nih.gov/22549953/)

71. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.* 2008; 7(1): 11–20. doi: [10.1016/j.cmet.2007.10.002](https://doi.org/10.1016/j.cmet.2007.10.002)
72. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009; 324(5930): 1029–1033. doi: [10.1126/science.1160809](https://doi.org/10.1126/science.1160809) PMID: [19460998](https://pubmed.ncbi.nlm.nih.gov/19460998/)
73. Hu J, Locasale JW, Bielas JH, O'Sullivan J, Sheahan K, Cantley LC, et al. Heterogeneity of tumor-induced gene expression changes in the human metabolic network. *Nat Biotechnol.* 2013; 31(6): 522–529. doi: [10.1038/nbt.2530](https://doi.org/10.1038/nbt.2530) PMID: [23604282](https://pubmed.ncbi.nlm.nih.gov/23604282/)
74. Gruss OJ, Vernos I. The mechanism of spindle assembly: functions of Ran and its target TPX2. *J Cell Biol.* 2004; 166(7): 949–955. doi: [10.1083/jcb.200312112](https://doi.org/10.1083/jcb.200312112) PMID: [15452138](https://pubmed.ncbi.nlm.nih.gov/15452138/)
75. Kalab P, Heald R. The RanGTP gradient—a GPS for the mitotic spindle. *J Cell Sci.* 2008; 121(Pt 10): 1577–1586. doi: [10.1242/jcs.005959](https://doi.org/10.1242/jcs.005959) PMID: [18469014](https://pubmed.ncbi.nlm.nih.gov/18469014/)
76. Bischoff FR, Krebber H, Smirnova E, Dong W, Ponstingl H. Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. *EMBO J.* 1995; 14(4): 705–715. PMID: [7882974](https://pubmed.ncbi.nlm.nih.gov/7882974/)
77. Zhang MS, Arnaoutov A, Dasso M. RanBP1 governs spindle assembly by defining mitotic Ran-GTP production. *Dev Cell.* 2014; 31(4): 393–404. doi: [10.1016/j.devcel.2014.10.014](https://doi.org/10.1016/j.devcel.2014.10.014) PMID: [25458009](https://pubmed.ncbi.nlm.nih.gov/25458009/)
78. Ems-McClung SC, Zheng Y, Walczak CE. Importin alpha/beta and Ran-GTP regulate XCTK2 microtubule binding through a bipartite nuclear localization signal. *Mol Biol Cell.* 2004; 15(1): 46–57. doi: [10.1091/mbc.E03-07-0454](https://doi.org/10.1091/mbc.E03-07-0454) PMID: [13679510](https://pubmed.ncbi.nlm.nih.gov/13679510/)
79. Nachury MV, Maresca TJ, Salmon WC, Waterman-Storer CM, Heald R, Weis K. Importin β Is a Mitotic Target of the Small GTPase Ran in Spindle Assembly. *Cell.* 2001; 104(1): 95–106. doi: [10.1016/S0092-8674\(01\)00194-5](https://doi.org/10.1016/S0092-8674(01)00194-5) PMID: [11163243](https://pubmed.ncbi.nlm.nih.gov/11163243/)
80. Wiese C, Wilde A, Moore MS, Adam SA, Merdes A, Zheng Y. Role of importin-beta in coupling Ran to downstream targets in microtubule assembly. *Science.* 2001; 291(5504): 653–656. PMID: [11229403](https://pubmed.ncbi.nlm.nih.gov/11229403/)
81. Trieselmann N, Armstrong S, Rauw J, Wilde A. Ran modulates spindle assembly by regulating a subset of TPX2 and Kid activities including Aurora A activation. *J Cell Sci.* 2003; 116(Pt 23): 4791–4798. doi: [10.1242/jcs.00798](https://doi.org/10.1242/jcs.00798) PMID: [14600264](https://pubmed.ncbi.nlm.nih.gov/14600264/)
82. Tanenbaum ME, Macurek L, Janssen A, Geers EF, Alvarez-Fernandez M, Medema RH. Kif15 cooperates with eg5 to promote bipolar spindle assembly. *Curr Biol.* 2009; 19(20): 1703–1711. doi: [10.1016/j.cub.2009.08.027](https://doi.org/10.1016/j.cub.2009.08.027) PMID: [19818618](https://pubmed.ncbi.nlm.nih.gov/19818618/)
83. Silk AD, Holland AJ, Cleveland DW. Requirements for NuMA in maintenance and establishment of mammalian spindle poles. *J Cell Biol.* 2009; 184(5): 677–690. doi: [10.1083/jcb.200810091](https://doi.org/10.1083/jcb.200810091) PMID: [19255246](https://pubmed.ncbi.nlm.nih.gov/19255246/)
84. Katayama H, Sasai K, Kloc M, Brinkley BR, Sen S. Aurora kinase-A regulates kinetochore/chromatin associated microtubule assembly in human cells. 2008; 7(17): 2691–2704.
85. Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. *Nat Rev Drug Discov.* 2012; 11(10): 790–811. doi: [10.1038/nrd3810](https://doi.org/10.1038/nrd3810) PMID: [23000686](https://pubmed.ncbi.nlm.nih.gov/23000686/)
86. Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF-beta receptor. *Nature.* 1994; 370(6488): 341–347. doi: [10.1038/370341a0](https://doi.org/10.1038/370341a0) PMID: [8047140](https://pubmed.ncbi.nlm.nih.gov/8047140/)
87. Hyytiainen M, Penttinen C, Keski-Oja J. Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. *Crit Rev Clin Lab Sci.* 2004; 41(3): 233–264. doi: [10.1080/10408360490460933](https://doi.org/10.1080/10408360490460933) PMID: [15307633](https://pubmed.ncbi.nlm.nih.gov/15307633/)
88. Nakao A, Imamura T, Souchelnytskyi S, Kawabata M, Ishisaki A, Oeda E, et al. TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4. *EMBO J.* 1997; 16(17): 5353–5362. doi: [10.1093/emboj/16.17.5353](https://doi.org/10.1093/emboj/16.17.5353) PMID: [9311995](https://pubmed.ncbi.nlm.nih.gov/9311995/)
89. Hayashi H, Abdollah S, Qiu Y, Cai J, Xu YY, Grinnell BW, et al. The MAD-related protein Smad7 associates with the TGFbeta receptor and functions as an antagonist of TGFbeta signaling. *Cell.* 1997; 89(7): 1165–1173. PMID: [9215638](https://pubmed.ncbi.nlm.nih.gov/9215638/)
90. Fukuchi M, Imamura T, Chiba T, Ebisawa T, Kawabata M, Tanaka K, et al. Ligand-dependent degradation of Smad3 by a ubiquitin ligase complex of ROC1 and associated proteins. *Mol Biol Cell.* 2001; 12(5): 1431–1443. PMID: [11359933](https://pubmed.ncbi.nlm.nih.gov/11359933/)
91. Chen CR, Kang Y, Siegel PM, Massague J. E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. *Cell.* 2002; 110(1): 19–32. PMID: [12150994](https://pubmed.ncbi.nlm.nih.gov/12150994/)
92. Kress TR, Sabo A, Amati B. MYC: connecting selective transcriptional control to global RNA production. *Nat Rev Cancer.* 2015; 15(10): 593–607. doi: [10.1038/nrc3984](https://doi.org/10.1038/nrc3984) PMID: [26383138](https://pubmed.ncbi.nlm.nih.gov/26383138/)

93. Feng XH, Lin X, Derynck R. Smad2, Smad3 and Smad4 cooperate with Sp1 to induce p15(Ink4B) transcription in response to TGF-beta. *EMBO J.* 2000; 19(19): 5178–5193. doi: [10.1093/emboj/19.19.5178](https://doi.org/10.1093/emboj/19.19.5178) PMID: [11013220](https://pubmed.ncbi.nlm.nih.gov/11013220/)
94. Black AR, Black JD, Azizkhan-Clifford J. Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. *J Cell Physiol.* 2001; 188(2): 143–160. doi: [10.1002/jcp.1111](https://doi.org/10.1002/jcp.1111) PMID: [11424081](https://pubmed.ncbi.nlm.nih.gov/11424081/)
95. Kim WY, Sharpless NE. The Regulation of INK4/ARF in Cancer and Aging. *Cell.* 2006; 127(2): 265–275. doi: [10.1016/j.cell.2006.10.003](https://doi.org/10.1016/j.cell.2006.10.003) PMID: [17055429](https://pubmed.ncbi.nlm.nih.gov/17055429/)
96. Luo J, Yu YP, Ciepły K, Lin F, DeFlavia P, Dhir R, et al. Gene expression analysis of prostate cancers. *Mol Carcinog.* 2002; 33(1): 25–35. doi: [10.1002/mc.10018](https://doi.org/10.1002/mc.10018) PMID: [11807955](https://pubmed.ncbi.nlm.nih.gov/11807955/)
97. Chetcuti A, Margan S, Mann S, Russell P, Handelsman D, Rogers J, et al. Identification of differentially expressed genes in organ-confined prostate cancer by gene expression array. *Prostate.* 2001; 47(2): 132–140. doi: [10.1002/pros.1056](https://doi.org/10.1002/pros.1056) PMID: [11340636](https://pubmed.ncbi.nlm.nih.gov/11340636/)
98. Zhao H, Ramos CF, Brooks JD, Peehl DM. Distinctive gene expression of prostatic stromal cells cultured from diseased versus normal tissues. *J Cell Physiol.* 2007; 210(1): 111–121. doi: [10.1002/jcp.20828](https://doi.org/10.1002/jcp.20828) PMID: [17044071](https://pubmed.ncbi.nlm.nih.gov/17044071/)
99. Xu J, Stolk JA, Zhang X, Silva SJ, Houghton RL, Matsumura M, et al. Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. *Cancer Res.* 2000; 60(6): 1677–1682. PMID: [10749139](https://pubmed.ncbi.nlm.nih.gov/10749139/)
100. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science.* 1999; 286(5439): 531–537. PMID: [10521349](https://pubmed.ncbi.nlm.nih.gov/10521349/)
101. Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang CH, Angelo M, et al. Multiclass cancer diagnosis using tumor gene expression signatures. *Proc Natl Acad Sci U S A.* 2001; 98(26): 15149–15154. doi: [10.1073/pnas.211566398](https://doi.org/10.1073/pnas.211566398) PMID: [11742071](https://pubmed.ncbi.nlm.nih.gov/11742071/)
102. Gregg JL, Brown KE, Mintz EM, Piontkivska H, Fraizer GC. Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection. *BMC Cancer.* 2010; 10: 165–2407-10-165. doi: [10.1186/1471-2407-10-165](https://doi.org/10.1186/1471-2407-10-165)
103. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, et al. PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res.* 2011; 17(20): 6563–6573. doi: [10.1158/1078-0432.CCR-11-1244](https://doi.org/10.1158/1078-0432.CCR-11-1244) PMID: [21878536](https://pubmed.ncbi.nlm.nih.gov/21878536/)
104. Chaux A, Peskoe SB, Gonzalez-Roibon N, Schultz L, Albadine R, Hicks J, et al. Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer. *Mod Pathol.* 2012; 25(11): 1543–1549. doi: [10.1038/modpathol.2012.104](https://doi.org/10.1038/modpathol.2012.104) PMID: [22684219](https://pubmed.ncbi.nlm.nih.gov/22684219/)
105. Zeng L, Rowland RG, Lele SM, Kyprianou N. Apoptosis incidence and protein expression of p53, TGF-β receptor II, p27Kip1, and Smad4 in benign, premalignant, and malignant human prostate 1 Accepted for publication 0, 2003. *Hum Pathol.* 2004; 35(3): 290–297. doi: [10.1016/j.humpath.2003.11.001](https://doi.org/10.1016/j.humpath.2003.11.001) PMID: [15017584](https://pubmed.ncbi.nlm.nih.gov/15017584/)
106. Aitchison AA, Veerakumarasivam A, Vias M, Kumar R, Hamdy FC, Neal DE, et al. Promoter methylation correlates with reduced Smad4 expression in advanced prostate cancer. *Prostate.* 2008; 68(6): 661–674. doi: [10.1002/pros.20730](https://doi.org/10.1002/pros.20730) PMID: [18213629](https://pubmed.ncbi.nlm.nih.gov/18213629/)
107. Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, et al. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Res.* 1999; 59(17): 4180–4184. PMID: [10485450](https://pubmed.ncbi.nlm.nih.gov/10485450/)
108. Vaarala MH, Porvari K, Kyllonen A, Lukkarinen O, Vihko P. The TMPRSS2 gene encoding transmembrane serine protease is overexpressed in a majority of prostate cancer patients: detection of mutated TMPRSS2 form in a case of aggressive disease. *Int J Cancer.* 2001; 94(5): 705–710. doi: [10.1002/ijc.1526](https://doi.org/10.1002/ijc.1526) PMID: [11745466](https://pubmed.ncbi.nlm.nih.gov/11745466/)
109. Lucas JM, Heinlein C, Kim T, Hernandez SA, Malik MS, True LD, et al. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov.* 2014; 4(11): 1310–1325. doi: [10.1158/2159-8290.CD-13-1010](https://doi.org/10.1158/2159-8290.CD-13-1010) PMID: [25122198](https://pubmed.ncbi.nlm.nih.gov/25122198/)
110. Dijkstra S, Mulders PF, Schalken JA. Clinical use of novel urine and blood based prostate cancer biomarkers: a review. *Clin Biochem.* 2014; 47(10–11): 889–896. doi: [10.1016/j.clinbiochem.2013.10.023](https://doi.org/10.1016/j.clinbiochem.2013.10.023) PMID: [24177197](https://pubmed.ncbi.nlm.nih.gov/24177197/)
111. Fleming WH, Hamel A, MacDonald R, Ramsey E, Pettigrew NM, Johnston B, et al. Expression of the c-Myc protooncogene in human prostate hyperplasia. *Cancer research.* 1986; 46(3): 1535–1538. PMID: [2417706](https://pubmed.ncbi.nlm.nih.gov/2417706/)

112. Buttyan R, Sawczuk IS, Benson MC, Siegal JD, Olsson CA. Enhanced expression of the c-myc proto-oncogene in high-grade human prostate cancers. *Prostate*. 1987; 11(4): 327–337. PMID: [2446300](#)
113. Koh CM, Bieberich CJ, Dang CV, Nelson WG, Yegnasubramanian S, De Marzo AM. MYC and Prostate Cancer. *Genes Cancer*. 2010; 1(6): 617–628. doi: [10.1177/1947601910379132](#) PMID: [21779461](#)
114. Hawksworth D, Ravindranath L, Chen Y, Furusato B, Sesterhenn IA, McLeod DG, et al. Overexpression of C-MYC oncogene in prostate cancer predicts biochemical recurrence. *Prostate Cancer Prostatic Dis*. 2010; 13(4): 311–315. doi: [10.1038/pcan.2010.31](#) PMID: [20820186](#)
115. Gurel B, Iwata T, Koh CM, Jenkins RB, Lan F, Van Dang C, et al. Nuclear MYC protein overexpression is an early alteration in human prostate carcinogenesis. *Mod Pathol*. 2008; 21(9): 1156–1167. doi: [10.1038/modpathol.2008.111](#) PMID: [18567993](#)
116. Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, et al. Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays. *Cancer Res*. 1999; 59(4): 803–806. PMID: [10029066](#)
117. Jenkins RB, Qian J, Lieber MM, Bostwick DG. Detection of c-myc oncogene amplification and chromosomal anomalies in metastatic prostatic carcinoma by fluorescence in situ hybridization. *Cancer Res*. 1997; 57(3): 524–531. PMID: [9012485](#)
118. Sato H, Minei S, Hachiya T, Yoshida T, Takimoto Y. Fluorescence in situ hybridization analysis of c-myc amplification in stage TNM prostate cancer in Japanese patients. *Int J Urol*. 2006; 13(6): 761–766. PMID: [16834657](#)
119. Yang YA, Yu J. EZH2, an epigenetic driver of prostate cancer. *Protein Cell*. 2013; 4(5): 331–341. doi: [10.1007/s13238-013-2093-2](#) PMID: [23636686](#)
120. Moul JW, Bettencourt M, Sesterhenn IA, Mostofi FK, McLeod DG, Srivastava S, et al. Protein expression of p53, bcl-2, and KI-67 (MIB-1) as prognostic biomarkers in patients with surgically treated, clinically localized prostate cancer. *Surgery*. 1996; 120(2): 159–167. doi: [10.1016/S0039-6060\(96\)80283-2](#) PMID: [8751578](#)
121. Johnson MI, Robinson MC, Marsh C, Robson CN, Neal DE, Hamdy FC. Expression of Bcl-2, Bax, and p53 in high-grade prostatic intraepithelial neoplasia and localized prostate cancer: relationship with apoptosis and proliferation. *Prostate*. 1998; 37(4): 223–229. doi: [10.1002/\(SICI\)1097-0045\(19981201\)37:4<223::AID-PROS3>3.0.CO;2-O](#) PMID: [9831218](#)
122. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LW, Hsieh JT, et al. Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res*. 1992; 52(24): 6940–6944. PMID: [1458483](#)
123. Apakama I, Robinson M, Walter N, Charlton R, Royds J, Fuller C, et al. bcl-2 overexpression combined with p53 protein accumulation correlates with hormone-refractory prostate cancer. *Br J Cancer*. 1996; 74(8): 1258–1262. doi: [10.1038/bjc.1996.526](#) PMID: [8883414](#)
124. Colombel M, Symmans F, Gil S, O'Toole KM, Chopin D, Benson M, et al. Detection of the apoptosis-suppressing oncoprotein bc1-2 in hormone-refractory human prostate cancers. *Am J Pathol*. 1993; 143(2): 390–400. PMID: [7688182](#)
125. Bubendorf L, Sauter G, Moch H, Jordan P, Blochlinger A, Gasser TC, et al. Prognostic significance of Bcl-2 in clinically localized prostate cancer. *Am J Pathol*. 1996; 148(5): 1557–1565. PMID: [8623924](#)
126. Mack D, Hacker GW, Hauser-Kronberger G, Frick J, Dietze O. Vasoactive Intestinal Polypeptide (VIP) and Neuropeptide Tyrosine (NPY) in Prostate Carcinoma. *Eur J Cancer*. 1997; 33(2): 317–318. doi: [10.1016/S0959-8049\(96\)00402-9](#) PMID: [9135509](#)
127. Ueda K, Tatsuguchi A, Saichi N, Toyama A, Tamura K, Furihata M, et al. Plasma low-molecular-weight proteome profiling identified neuropeptide-Y as a prostate cancer biomarker polypeptide. *J Proteome Res*. 2013; 12(10): 4497–4506. doi: [10.1021/pr400547s](#) PMID: [23991666](#)
128. Liu A, Furusato B, Ravindranath L, Chen Y, Srikantan V, McLeod DG, et al. Quantitative analysis of a panel of gene expression in prostate cancer—with emphasis on NPY expression analysis. 2007; 8(12): 853–859.
129. Ruscica M, Dozio E, Boghossian S, Bovo G, Martos Riano V, Motta M, et al. Activation of the Y1 receptor by neuropeptide Y regulates the growth of prostate cancer cells. *Endocrinology*. 2006; 147(3): 1466–1473. PMID: [16339211](#)
130. Vainio P, Gupta S, Ketola K, Mirtti T, Mpindi JP, Kohonen P, et al. Arachidonic acid pathway members PLA2G7, HPGD, EPHX2, and CYP4F8 identified as putative novel therapeutic targets in prostate cancer. *Am J Pathol*. 2011; 178(2): 525–536. doi: [10.1016/j.ajpath.2010.10.002](#) PMID: [21281786](#)
131. Vainio P, Lehtinen L, Mirtti T, Hilvo M, Seppanen-Laakso T, Virtanen J, et al. Phospholipase PLA2G7, associated with aggressive prostate cancer, promotes prostate cancer cell migration and invasion and is inhibited by statins. *Oncotarget*. 2011; 2(12): 1176–1190. PMID: [22202492](#)

132. Zhong W, Peng J, He H, Wu D, Han Z, Bi X, et al. Ki-67 and PCNA expression in prostate cancer and benign prostatic hyperplasia. *Clin Invest Med*. 2008; 31(1): E8–E15. PMID: [18312749](#)
133. Thompson SJ, Mellon K, Charlton RG, Marsh C, Robinson M, Neal DE. P53 and Ki-67 immunoreactivity in human prostate cancer and benign hyperplasia. *Br J Urol*. 1992; 69(6): 609–613. PMID: [1379102](#)
134. Mucci NR, Rubin MA, Strawderman MS, Montie JE, Smith DC, Pienta KJ. Expression of nuclear antigen Ki-67 in prostate cancer needle biopsy and radical prostatectomy specimens. *J Natl Cancer Inst*. 2000; 92(23): 1941–1942. PMID: [11106686](#)
135. Revelos K, Petraki C, Gregorakis A, Scorilas A, Papanastasiou P, Tenta R, et al. p27(kip1) and Ki-67 (MIB1) immunohistochemical expression in radical prostatectomy specimens of patients with clinically localized prostate cancer. *In Vivo*. 2005; 19(5): 911–920. PMID: [16097446](#)
136. Grover SK, Agarwal S, Gupta S, Wadhwa N, Sharma N. Expression of Estrogen Receptor β and Ki 67 in Benign & Malignant Human Prostate Lesions by Immunohistochemistry. 2015; 21(3): 651–657. doi: [10.1007/s12253-014-9870-y](#)
137. Reis S, Timoszczuk L, Pontes-Junior J, Viana N, Silva I, Dip N, et al. The role of micro RNAs let7c, 100 and 218 expression and their target RAS, C-MYC, BUB1, RB, SMARCA5, LAMB3 and Ki-67 in prostate cancer. 2013; 68(5): 652–657.
138. Tamboli P, Amin MB, Schultz DS, Linden MD, Kubus J. Comparative analysis of the nuclear proliferative index (Ki-67) in benign prostate, prostatic intraepithelial neoplasia, and prostatic carcinoma. *Mod Pathol*. 1996; 9(10): 1015–1019. PMID: [8902840](#)
139. Van der Kwast TH. Prognostic prostate tissue biomarkers of potential clinical use. *Virchows Arch*. 2014; 464(3): 293–300. doi: [10.1007/s00428-014-1540-7](#) PMID: [24487790](#)
140. Remo A, Pancione M, Zanella C, Manfrin E. p16 Expression in Prostate Cancer and Nonmalignant Lesions: Novel Findings and Review of the Literature. *Appl Immunohistochem Mol Morphol*. 2015; doi: [10.1097/PAI.0000000000000171](#)
141. Royuela M, De Miguel MP, Bethencourt FR, Fraile B, Arenas MI, Paniagua R. IL-2, its receptors, and bcl-2 and bax genes in normal, hyperplastic and carcinomatous human prostates: immunohistochemical comparative analysis. *Growth Factors*. 2000; 18(2): 135–146. PMID: [11019784](#)
142. Royuela M, De Miguel MP, Bethencourt FR, Sanchez-Chapado M, Fraile B, Paniagua R. Transforming growth factor beta 1 and its receptor types I and II. Comparison in human normal prostate, benign prostatic hyperplasia, and prostatic carcinoma. *Growth Factors*. 1998; 16(2): 101–110. PMID: [9932228](#)
143. Cardillo MR, Petrangeli E, Perracchio L, Salvatori L, Ravenna L, Di Silverio F. Transforming growth factor-beta expression in prostate neoplasia. *Anal Quant Cytol Histol*. 2000; 22(1): 1–10. PMID: [10696454](#)
144. Shariat SF, Menesses-Diaz A, Kim IY, Muramoto M, Wheeler TM, Slawin KM. Tissue expression of transforming growth factor-beta1 and its receptors: correlation with pathologic features and biochemical progression in patients undergoing radical prostatectomy. *Urology*. 2004; 63(6): 1191–1197. doi: [10.1016/j.urology.2003.12.015](#) PMID: [15183988](#)
145. Perry KT, Anthony CT, Steiner MS. Immunohistochemical localization of TGF beta 1, TGF beta 2, and TGF beta 3 in normal and malignant human prostate. *Prostate*. 1997; 33(2): 133–140. doi: [10.1002/\(SICI\)1097-0045\(19971001\)33:2<133::AID-PROS7>3.0.CO;2-L](#) PMID: [9316654](#)
146. Gerdes MJ, Larsen M, McBride L, Dang TD, Lu B, Rowley DR. Localization of transforming growth factor-beta1 and type II receptor in developing normal human prostate and carcinoma tissues. *J Histochem Cytochem*. 1998; 46(3): 379–388. PMID: [9487120](#)
147. Truong LD, Kadmon D, McCune BK, Flanders KC, Scardino PT, Thompson TC. Association of transforming growth factor- β 1 with prostate cancer: An immunohistochemical study. *Hum Pathol*. 1993; 24(1): 4–9. doi: [10.1016/0046-8177\(93\)90055-L](#) PMID: [7678092](#)
148. Djonov V, Ball RK, Graf S, Mottaz AE, Arnold AM, Flanders K, et al. Transforming growth factor-beta 3 is expressed in nondividing basal epithelial cells in normal human prostate and benign prostatic hyperplasia, and is no longer detectable in prostate carcinoma. *Prostate*. 1997; 31(2): 103–109. doi: [10.1002/\(SICI\)1097-0045\(19970501\)31:2<103::AID-PROS5>3.0.CO;2-O](#) PMID: [9140123](#)
149. Twillie DA, Eisenberger MA, Carducci MA, Hseih W, Kim WY, Simons JW. Interleukin-6: A candidate mediator of human prostate cancer morbidity. *Urology*. 1995; 45(3): 542–549. doi: [10.1016/S0090-4295\(99\)80034-X](#) PMID: [7879350](#)
150. Hobisch A, Rogatsch H, Hittmair A, Fuchs D, Bartsch G Jr, Klocker H, et al. Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. *J Pathol*. 2000; 191(3): 239–244. doi: [10.1002/1096-9896\(2000\)9999:9999::AID-PATH633>3.0.CO;2-X](#) PMID: [10878544](#)

151. Park JI, Lee MG, Cho K, Park BJ, Chae KS, Byun DS, et al. Transforming growth factor-beta1 activates interleukin-6 expression in prostate cancer cells through the synergistic collaboration of the Smad2, p38-NF-kappaB, JNK, and Ras signaling pathways. *Oncogene*. 2003; 22(28): 4314–4332. doi: [10.1038/sj.onc.1206478](https://doi.org/10.1038/sj.onc.1206478) PMID: [12853969](https://pubmed.ncbi.nlm.nih.gov/12853969/)
152. Bouraoui Y, Ricote M, Garcia-Tunon I, Rodriguez-Berriguete G, Touffehi M, Rais NB, et al. Pro-inflammatory cytokines and prostate-specific antigen in hyperplasia and human prostate cancer. *Cancer Detect Prev*. 2008; 32(1): 23–32. doi: [10.1016/j.cdp.2008.02.007](https://doi.org/10.1016/j.cdp.2008.02.007) PMID: [18400418](https://pubmed.ncbi.nlm.nih.gov/18400418/)
153. Mechergui YB, Ben Jemaa A, Mezigh C, Fraile B, Ben Rais N, Paniagua R, et al. The profile of prostate epithelial cytokines and its impact on sera prostate specific antigen levels. *Inflammation*. 2009; 32(3): 202–210. doi: [10.1007/s10753-009-9121-7](https://doi.org/10.1007/s10753-009-9121-7) PMID: [19399601](https://pubmed.ncbi.nlm.nih.gov/19399601/)
154. Lee SO, Lou W, Hou M, de Miguel F, Gerber L, Gao AC. Interleukin-6 promotes androgen-independent growth in LNCaP human prostate cancer cells. *Clin Cancer Res*. 2003; 9(1): 370–376. PMID: [12538490](https://pubmed.ncbi.nlm.nih.gov/12538490/)
155. D E Drachenberg A.-A.A. Elgamal, R Rowbotham, M Peterson, G P Murphy. Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. *The Prostate*. 1999; 41(2): 127–133.
156. Akimoto S, Okumura A, Fuse H. Relationship between serum levels of interleukin-6, tumor necrosis factor- α and bone turnover markers in prostate cancer patients. *Endocrine journal*. 1998; 45(2): 183–189. PMID: [9700471](https://pubmed.ncbi.nlm.nih.gov/9700471/)
157. Adler HL, Mccurdy MA, Kattan MW, Timme TL, Scardino PT, Thompson TC. Elevated Levels of Circulating Interleukin-6 and Transforming Growth Factor-Beta 1 in Patients with Metastatic Prostatic Carcinoma. *J Urol*. 1999; 161(1): 182–187. doi: [10.1016/S0022-5347\(01\)62092-5](https://doi.org/10.1016/S0022-5347(01)62092-5) PMID: [10037394](https://pubmed.ncbi.nlm.nih.gov/10037394/)
158. Wise GJ, Marella VK, Talluri G, Shirazian D. Cytokine variations in patients with hormone treated prostate cancer. *J Urol*. 2000; 164(3 Pt 1): 722–725. PMID: [10953133](https://pubmed.ncbi.nlm.nih.gov/10953133/)
159. Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. *Urology*. 2001; 58(6): 1008–1015. doi: [10.1016/S0090-4295\(01\)01405-4](https://doi.org/10.1016/S0090-4295(01)01405-4) PMID: [11744478](https://pubmed.ncbi.nlm.nih.gov/11744478/)
160. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF- α correlate with clinicopathological features and patient survival in patients with prostate cancer. *Br J Cancer*. 2004; 90(12): 2312–2316. doi: [10.1038/sj.bjc.6601814](https://doi.org/10.1038/sj.bjc.6601814) PMID: [15150588](https://pubmed.ncbi.nlm.nih.gov/15150588/)
161. George DJ, Halabi S, Shepard TF, Sanford B, Vogelzang NJ, Small EJ, et al. The prognostic significance of plasma interleukin-6 levels in patients with metastatic hormone-refractory prostate cancer: results from cancer and leukemia group B 9480. *Clin Cancer Res*. 2005; 11(5): 1815–1820. PMID: [15756004](https://pubmed.ncbi.nlm.nih.gov/15756004/)
162. Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. *Clin Cancer Res*. 2000; 6(7): 2702–2706. PMID: [10914713](https://pubmed.ncbi.nlm.nih.gov/10914713/)
163. Ricote M, Garcia-Tunon I, Bethencourt FR, Fraile B, Paniagua R, Royuela M. Interleukin-1 (IL-1 α and IL-1 β) and its receptors (IL-1RI, IL-1RII, and IL-1Ra) in prostate carcinoma. *Cancer*. 2004; 100(7): 1388–1396. doi: [10.1002/cncr.20142](https://doi.org/10.1002/cncr.20142) PMID: [15042672](https://pubmed.ncbi.nlm.nih.gov/15042672/)
164. Liu Q, Russell MR, Shahriari K, Jernigan DL, Lioni MI, Garcia FU, et al. Interleukin-1 Promotes Skeletal Colonization and Progression of Metastatic Prostate Cancer Cells with Neuroendocrine Features. *Cancer Res*. 2013; 73(11): 3297–3305. doi: [10.1158/0008-5472.CAN-12-3970](https://doi.org/10.1158/0008-5472.CAN-12-3970) PMID: [23536554](https://pubmed.ncbi.nlm.nih.gov/23536554/)
165. Aaltomaa S, Lipponen P, Eskelinen M, Ala-Opas M, Kosma VM. Prognostic value and expression of p21(waf1/cip1) protein in prostate cancer. *Prostate*. 1999; 39(1): 8–15. doi: [10.1002/\(SICI\)1097-0045\(19990401\)39:1<8::AID-PROS2>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-0045(19990401)39:1<8::AID-PROS2>3.0.CO;2-N) PMID: [10221260](https://pubmed.ncbi.nlm.nih.gov/10221260/)
166. Omar EA, Behloul H, Chevalier S, Aprikian AG. Relationship of p21 (WAF-1) protein expression with prognosis in advanced prostate cancer treated by androgen ablation. *Prostate*. 2001; 49(3): 191–199. doi: [10.1002/pros.1134](https://doi.org/10.1002/pros.1134) PMID: [11746264](https://pubmed.ncbi.nlm.nih.gov/11746264/)
167. Rigaud J, Tiguert R, Decobert M, Hovington H, Latulippe E, Laverdiere J, et al. Expression of p21 cell cycle protein is an independent predictor of response to salvage radiotherapy after radical prostatectomy. *Prostate*. 2004; 58(3): 269–276. doi: [10.1002/pros.10329](https://doi.org/10.1002/pros.10329) PMID: [14743466](https://pubmed.ncbi.nlm.nih.gov/14743466/)
168. Gotoh A, Kao C, Ko S, Hamada K, Liu T, Chung LWK. Cytotoxic Effects of Recombinant Adenovirus p53 and Cell Cycle Regulator Genes (p21 sup WAF1/CIP1 and p16 sup CDKN4) in Human Prostate Cancers. *J Urol*. 1997; 158(2): 636–641. doi: [10.1016/S0022-5347\(01\)64574-9](https://doi.org/10.1016/S0022-5347(01)64574-9) PMID: [9224383](https://pubmed.ncbi.nlm.nih.gov/9224383/)
169. Green MM, Hiley CT, Shanks JH, Bottomley IC, West CM, Cowan RA, et al. Expression of vascular endothelial growth factor (VEGF) in locally invasive prostate cancer is prognostic for radiotherapy outcome. *Int J Radiat Oncol Biol Phys*. 2007; 67(1): 84–90. PMID: [17189065](https://pubmed.ncbi.nlm.nih.gov/17189065/)

170. Mori R, Dorff TB, Xiong S, Tarabolous CJ, Ye W, Groshen S, et al. The relationship between proangiogenic gene expression levels in prostate cancer and their prognostic value for clinical outcomes. *Prostate*. 2010; 70(15): 1692–1700. doi: [10.1002/pros.21204](https://doi.org/10.1002/pros.21204) PMID: [20564320](https://pubmed.ncbi.nlm.nih.gov/20564320/)
171. Anees M, Horak P, El-Gazzar A, Susani M, Heinze G, Perco P, et al. Recurrence-free survival in prostate cancer is related to increased stromal TRAIL expression. *Cancer*. 2011; 117(6): 1172–1182. doi: [10.1002/ncr.25504](https://doi.org/10.1002/ncr.25504) PMID: [21381010](https://pubmed.ncbi.nlm.nih.gov/21381010/)
172. Sanlioglu AD, Koksali IT, Ciftcioglu A, Baykara M, Luleci G, Sanlioglu S. Differential Expression of TRAIL and its Receptors in Benign and Malignant Prostate Tissues. *J Urol*. 2007; 177(1): 359–364. doi: [10.1016/j.juro.2006.08.087](https://doi.org/10.1016/j.juro.2006.08.087) PMID: [17162091](https://pubmed.ncbi.nlm.nih.gov/17162091/)
173. Nunez C, Cansino JR, Bethencourt F, Perez-Utrilla M, Fraile B, Martinez-Onsurbe P, et al. TNF/IL-1/NIK/NF-kappa B transduction pathway: a comparative study in normal and pathological human prostate (benign hyperplasia and carcinoma). *Histopathology*. 2008; 53(2): 166–176. doi: [10.1111/j.1365-2559.2008.03092.x](https://doi.org/10.1111/j.1365-2559.2008.03092.x) PMID: [18752500](https://pubmed.ncbi.nlm.nih.gov/18752500/)
174. Zhang M, Zhang L, Zou J, Yao C, Xiao H, Liu Q, et al. Evaluating reproducibility of differential expression discoveries in microarray studies by considering correlated molecular changes. *Bioinformatics*. 2009; 25(13): 1662–1668. doi: [10.1093/bioinformatics/btp295](https://doi.org/10.1093/bioinformatics/btp295) PMID: [19417058](https://pubmed.ncbi.nlm.nih.gov/19417058/)
175. Moore JD. The Ran-GTPase and cell-cycle control. *Bioessays*. 2001; 23(1): 77–85. doi: [10.1002/1521-1878\(200101\)23:1<77::AID-BIES1010>3.0.CO;2-E](https://doi.org/10.1002/1521-1878(200101)23:1<77::AID-BIES1010>3.0.CO;2-E) PMID: [11135312](https://pubmed.ncbi.nlm.nih.gov/11135312/)
176. Clarke PR, Zhang C. Spatial and temporal coordination of mitosis by Ran GTPase. *Nat Rev Mol Cell Biol*. 2008; 9(6): 464–477. doi: [10.1038/nrm2410](https://doi.org/10.1038/nrm2410) PMID: [18478030](https://pubmed.ncbi.nlm.nih.gov/18478030/)
177. Abe H, Kamai T, Shirataka H, Oyama T, Arai K, Yoshida K. High expression of Ran GTPase is associated with local invasion and metastasis of human clear cell renal cell carcinoma. *Int J Cancer*. 2008; 122(10): 2391–2397. doi: [10.1002/ijc.23400](https://doi.org/10.1002/ijc.23400) PMID: [18241036](https://pubmed.ncbi.nlm.nih.gov/18241036/)
178. Ouellet V, Provencher DM, Maugard CM, Le Page C, Ren F, Lussier C, et al. Discrimination between serous low malignant potential and invasive epithelial ovarian tumors using molecular profiling. *Oncogene*. 2005; 24(29): 4672–4687. PMID: [15940270](https://pubmed.ncbi.nlm.nih.gov/15940270/)
179. Ouellet V, Guyot MC, Le Page C, Filali-Mouhim A, Lussier C, Tonin PN, et al. Tissue array analysis of expression microarray candidates identifies markers associated with tumor grade and outcome in serous epithelial ovarian cancer. *Int J Cancer*. 2006; 119(3): 599–607. doi: [10.1002/ijc.21902](https://doi.org/10.1002/ijc.21902) PMID: [16572426](https://pubmed.ncbi.nlm.nih.gov/16572426/)
180. Hung KE, Faca V, Song K, Sarracino DA, Richard LG, Krastins B, et al. Comprehensive proteome analysis of an Apc mouse model uncovers proteins associated with intestinal tumorigenesis. *Cancer Prev Res (Phila)*. 2009; 2(3): 224–233. doi: [10.1158/1940-6207.CAPR-08-0153](https://doi.org/10.1158/1940-6207.CAPR-08-0153)
181. Xia F, Lee CW, Altieri DC. Tumor cell dependence on Ran-GTP-directed mitosis. *Cancer Res*. 2008; 68(6): 1826–1833. doi: [10.1158/0008-5472.CAN-07-5279](https://doi.org/10.1158/0008-5472.CAN-07-5279) PMID: [18339863](https://pubmed.ncbi.nlm.nih.gov/18339863/)
182. Azuma K, Sasada T, Takedatsu H, Shomura H, Koga M, Maeda Y, et al. Ran, a small GTPase gene, encodes cytotoxic T lymphocyte (CTL) epitopes capable of inducing HLA-A33-restricted and tumor-reactive CTLs in cancer patients. *Clin Cancer Res*. 2004; 10(19): 6695–6702. PMID: [15475460](https://pubmed.ncbi.nlm.nih.gov/15475460/)
183. Barres V, Ouellet V, Lafontaine J, Tonin PN, Provencher DM, Mes-Masson AM. An essential role for Ran GTPase in epithelial ovarian cancer cell survival. *Mol Cancer*. 2010; 9: 272. doi: [10.1186/1476-4598-9-272](https://doi.org/10.1186/1476-4598-9-272) PMID: [20942967](https://pubmed.ncbi.nlm.nih.gov/20942967/)
184. Kurisetty VV, Johnston PG, Johnston N, Erwin P, Crowe P, Fernig DG, et al. RAN GTPase is an effector of the invasive/metastatic phenotype induced by osteopontin. *Oncogene*. 2008; 27(57): 7139–7149. doi: [10.1038/onc.2008.325](https://doi.org/10.1038/onc.2008.325) PMID: [18794800](https://pubmed.ncbi.nlm.nih.gov/18794800/)
185. Katayama H, Brinkley WR, Sen S. The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev*. 2003; 22(4): 451–464. PMID: [12884918](https://pubmed.ncbi.nlm.nih.gov/12884918/)
186. Vainio P, Mpindi JP, Kohonen P, Fey V, Mirtti T, Alanen KA, et al. High-throughput transcriptomic and RNAi analysis identifies AIM1, ERGIC1, TMED3 and TPX2 as potential drug targets in prostate cancer. *PLoS One*. 2012; 7(6): e39801. doi: [10.1371/journal.pone.0039801](https://doi.org/10.1371/journal.pone.0039801) PMID: [22761906](https://pubmed.ncbi.nlm.nih.gov/22761906/)
187. Neumayer G, Belzil C, Gruss OJ, Nguyen MD. TPX2: of spindle assembly, DNA damage response, and cancer. *Cell Mol Life Sci*. 2014; 71(16): 3027–3047. doi: [10.1007/s00018-014-1582-7](https://doi.org/10.1007/s00018-014-1582-7) PMID: [24556998](https://pubmed.ncbi.nlm.nih.gov/24556998/)
188. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet*. 2006; 38(9): 1043–1048. PMID: [16921376](https://pubmed.ncbi.nlm.nih.gov/16921376/)
189. Pannu V, Rida PC, Ogden A, Turaga RC, Donthamsetty S, Bowen NJ, et al. HSET overexpression fuels tumor progression via centrosome clustering-independent mechanisms in breast cancer patients. *Oncotarget*. 2015; 6(8): 6076–6091. PMID: [25788277](https://pubmed.ncbi.nlm.nih.gov/25788277/)

190. Li P, Yu X, Ge K, Melamed J, Roeder RG, Wang Z. Heterogeneous Expression and Functions of Androgen Receptor Co-Factors in Primary Prostate Cancer. 2002; 161(4): 1467–1474. doi: [10.1016/S0002-9440\(10\)64422-7](https://doi.org/10.1016/S0002-9440(10)64422-7)
191. Hsiao P-, Lin D-, Nakao R, Chang C. The Linkage of Kennedy's Neuron Disease to ARA24, the First Identified Androgen Receptor Polyglutamine Region-associated Coactivator. *J Biol Chem.* 1999; 274(29): 20229–20234. doi: [10.1074/jbc.274.29.20229](https://doi.org/10.1074/jbc.274.29.20229) PMID: [10400640](https://pubmed.ncbi.nlm.nih.gov/10400640/)
192. Harada N, Ohmori Y, Yamaji R, Higashimura Y, Okamoto K, Isohashi F, et al. ARA24/Ran enhances the androgen-dependent NH₂- and COOH-terminal interaction of the androgen receptor. *Biochem Biophys Res Commun.* 2008; 373(3): 373–377. doi: [10.1016/j.bbrc.2008.06.024](https://doi.org/10.1016/j.bbrc.2008.06.024) PMID: [18565325](https://pubmed.ncbi.nlm.nih.gov/18565325/)
193. Heldin CH, Landstrom M, Moustakas A. Mechanism of TGF-beta signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition. *Curr Opin Cell Biol.* 2009; 21(2): 166–176. doi: [10.1016/j.ceb.2009.01.021](https://doi.org/10.1016/j.ceb.2009.01.021) PMID: [19237272](https://pubmed.ncbi.nlm.nih.gov/19237272/)
194. Blobbe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med.* 2000; 342(18): 1350–1358. doi: [10.1056/NEJM200005043421807](https://doi.org/10.1056/NEJM200005043421807) PMID: [10793168](https://pubmed.ncbi.nlm.nih.gov/10793168/)
195. Massague J. TGFbeta in Cancer. *Cell.* 2008; 134(2): 215–230. doi: [10.1016/j.cell.2008.07.001](https://doi.org/10.1016/j.cell.2008.07.001) PMID: [18662538](https://pubmed.ncbi.nlm.nih.gov/18662538/)
196. Ravitz MJ, Wenner CE. Cyclin-dependent kinase regulation during G1 phase and cell cycle regulation by TGF-beta. *Adv Cancer Res.* 1997; 71: 165–207. PMID: [9111866](https://pubmed.ncbi.nlm.nih.gov/9111866/)
197. Aalinkeel R, Nair MP, Sufrin G, Mahajan SD, Chadha KC, Chawda RP, et al. Gene expression of angiogenic factors correlates with metastatic potential of prostate cancer cells. *Cancer Res.* 2004; 64(15): 5311–5321. doi: [10.1158/0008-5472.CAN-2506-2](https://doi.org/10.1158/0008-5472.CAN-2506-2) PMID: [15289337](https://pubmed.ncbi.nlm.nih.gov/15289337/)
198. Wan Y, Yang M, Kolattukudy S, Stark GR, Lu T. Activation of cAMP-responsive-element-binding protein by PI3 kinase and p38 MAPK is essential for elevated expression of transforming growth factor beta2 in cancer cells. *J Interferon Cytokine Res.* 2010; 30(9): 677–681. doi: [10.1089/jir.2009.0117](https://doi.org/10.1089/jir.2009.0117) PMID: [20629536](https://pubmed.ncbi.nlm.nih.gov/20629536/)
199. Dallas SL, Zhao S, Cramer SD, Chen Z, Peehl DM, Bonewald LF. Preferential production of latent transforming growth factor beta-2 by primary prostatic epithelial cells and its activation by prostate-specific antigen. *J Cell Physiol.* 2005; 202(2): 361–370. doi: [10.1002/jcp.20147](https://doi.org/10.1002/jcp.20147) PMID: [15389580](https://pubmed.ncbi.nlm.nih.gov/15389580/)
200. Kim IY, Ahn HJ, Zelner DJ, Shaw JW, Lang S, Kato M, et al. Loss of expression of transforming growth factor beta type I and type II receptors correlates with tumor grade in human prostate cancer tissues. *Clin Cancer Res.* 1996; 2(8): 1255–1261. PMID: [9816295](https://pubmed.ncbi.nlm.nih.gov/9816295/)
201. Kim IY, Ahn HJ, Lang S, Oefelein MG, Oyasu R, Kozlowski JM, et al. Loss of expression of transforming growth factor-beta receptors is associated with poor prognosis in prostate cancer patients. *Clin Cancer Res.* 1998; 4(7): 1625–1630. PMID: [9676836](https://pubmed.ncbi.nlm.nih.gov/9676836/)
202. Wikstrom P, Stattin P, Franck-Lissbrant I, Damber JE, Bergh A. Transforming growth factor beta 1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer. *Prostate.* 1998; 37(1): 19–29. doi: [10.1002/\(SICI\)1097-0045\(19980915\)37:1<19::AID-PROS4>3.0.CO;2-3](https://doi.org/10.1002/(SICI)1097-0045(19980915)37:1<19::AID-PROS4>3.0.CO;2-3) PMID: [9721065](https://pubmed.ncbi.nlm.nih.gov/9721065/)
203. Shariat SF, Kattan MW, Traxel E, Andrews B, Zhu K, Wheeler TM, et al. Association of pre- and post-operative plasma levels of transforming growth factor beta(1) and interleukin 6 and its soluble receptor with prostate cancer progression. *Clin Cancer Res.* 2004; 10(6): 1992–1999. PMID: [15041717](https://pubmed.ncbi.nlm.nih.gov/15041717/)
204. Ajiboye S, Sissung TM, Sharifi N, Figg WD. More than an accessory: implications of type III transforming growth factor-beta receptor loss in prostate cancer. *BJU Int.* 2010; 105(7): 913–916. doi: [10.1111/j.1464-410X.2009.08999.x](https://doi.org/10.1111/j.1464-410X.2009.08999.x) PMID: [20067462](https://pubmed.ncbi.nlm.nih.gov/20067462/)
205. Lucia MS, Sporn MB, Roberts AB, Stewart LV, Danielpour D. The role of transforming growth factor-beta1, -beta2, and -beta3 in androgen-responsive growth of NRP-152 rat prostatic epithelial cells. *J Cell Physiol.* 1998; 175(2): 184–192. doi: [10.1002/\(SICI\)1097-4652\(199805\)175:2<184::AID-JCP8>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-4652(199805)175:2<184::AID-JCP8>3.0.CO;2-K) PMID: [9525477](https://pubmed.ncbi.nlm.nih.gov/9525477/)
206. Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res.* 2009; 19(1): 128–139. doi: [10.1038/cr.2008.328](https://doi.org/10.1038/cr.2008.328) PMID: [19114990](https://pubmed.ncbi.nlm.nih.gov/19114990/)
207. Zavadil J, Bitzer M, Liang D, Yang YC, Massimi A, Kneitz S, et al. Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. *Proc Natl Acad Sci U S A.* 2001; 98(12): 6686–6691. doi: [10.1073/pnas.111614398](https://doi.org/10.1073/pnas.111614398) PMID: [11390996](https://pubmed.ncbi.nlm.nih.gov/11390996/)
208. Savli H, Szendroi A, Romics I, Nagy B. Gene network and canonical pathway analysis in prostate cancer: a microarray study. *Exp Mol Med.* 2008; 40(2): 176–185. PMID: [18446056](https://pubmed.ncbi.nlm.nih.gov/18446056/)
209. Jia P, Liu Y, Zhao Z. Integrative pathway analysis of genome-wide association studies and gene expression data in prostate cancer. *BMC Syst Biol.* 2012; 6 Suppl 3: S3–S13. doi: [10.1186/1752-0509-6-S3-S13](https://doi.org/10.1186/1752-0509-6-S3-S13) PMID: [23281744](https://pubmed.ncbi.nlm.nih.gov/23281744/)