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Original Article

Genome and transcriptome profiling of *FBXW* family in human prostate cancer

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Abstract: F-box and WD repeat domain containing (FBXW) family of E3 ligases has 10 members that ubiquitinate substrate proteins for proteasome-mediated degradation. Publicly archived datasets from The Cancer Genome Atlas (TCGA), Prostate Cancer Transcriptome Atlas (PCTA), and cBioPortal were analyzed for mRNA expression and genetic alterations of 10 *FBXW* genes. We found that *FBXW7* mRNA expression was significantly decreased in primary prostate cancers compared to normal prostate tissues, whereas mRNA expression of *FBXW8-10* was significantly increased in primary prostate cancers compared to normal prostate tissues. *FBXW7* mRNA expression was also significantly decreased in breast invasive carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, lung squamous cell carcinoma, and uterine corpus endometrial carcinoma. In contrast, *FBXW7* mRNA expression was significantly increased in cholangiocarcinoma, colon adenocarcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, pheochromocytoma and paraganglioma, and thyroid carcinoma. Compared to normal tissues, *FBXW5* mRNA expression was significantly increased in breast invasive carcinoma, cholangiocarcinoma, kidney chromophobe, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, thyroid carcinoma, and uterine corpus endometrial carcinoma, whereas *FBXW5* mRNA expression was only significantly decreased in colon adenocarcinoma. There were not any significant differences in gene copy number gains, losses, or gene simple somatic mutations between primary prostate cancers and normal prostate tissues. The mRNA expression levels of *FBXW5, 7, 8, 9*, and *12* were significantly higher in metastatic castration-resistant prostate cancers (mCRPCs) than primary prostate cancers, whereas mRNA expression levels of *FBXW1* and *4* were significantly lower in mCRPCs than primary prostate cancers. All 10 *FBXW* genes had significantly more overall gene alterations including gene amplifications in mCRPCs than primary prostate cancers. *FBXW5* and *7* had significantly more gene deep deletions in mCRPCs than primary prostate cancers and *FBXW7* had significantly more gene missense mutations in mCRPCs than primary prostate cancers. Our findings suggest that different *FBXW* genes have differential mRNA expression in prostate cancer and other cancer types and their gene amplifications are significantly more in mCRPCs than primary prostate cancers. *FBXW7* mRNA expression is consistently decreased in primary prostate cancers compared to normal prostate tissues.

Keywords: Prostate cancer, mCRPC, *FBXW*, gene amplification

Introduction

Prostate cancer is the most common malignancy and the second most common cause of cancer-related deaths in American men; the American Cancer Society estimates that there will be approximately 191,930 new cases and 33,330 deaths due to prostate cancer in 2020 [3]. The etiology of prostate cancer is not clear, but age, race, and family history are well-known risk factors. Environmental factors and epigenetic as well as genetic alterations have also

been demonstrated to play important roles in prostate cancer initiation and progression [4]. Distant metastases were found in 35% of prostate cancer patients at autopsies, with the most frequent metastatic sites at the bone (90%), lungs (46%), liver (25%), pleura (21%), and adrenal glands (13%) [5]. Metastatic prostate cancers are usually treated with androgen deprivation therapy (ADT) and they often respond well as they are mostly hormone-naïve prostate cancers. However, almost all of them eventually become insensitive to ADT even when the blood levels of androgens are at castration levels, thus becoming metastatic castration-resistant prostate cancers (mCRPCs).

The F-box is a protein motif of approximately 50 amino acids that mediates protein-protein interactions. F-box protein, S-phase kinase-associated protein 1 (Skp1), Cullin 1 (CUL1), and RING-box protein 1 (RBX1, also known as Regulator of Cullins 1, ROC1) form the SCF ubiquitin-ligase complexes (named after the main components Skp1, CUL1, and F-box protein), which bind to substrate proteins for ubiquitination-mediated proteolysis. F-box proteins are functionally conserved from budding yeast, Caenorhabditis elegans, Drosophila, to humans [6]. In the human genome, 69 putative F-box proteins have been identified, which may form a variety of different SCF complexes to target a wide range of proteins for degradation [7]. F-box proteins are subclassified into three families largely based on three recognizable domains: WD repeats, leucine-rich repeats (LRR), and other types of protein interaction domains. The F-box and WD repeat domain containing (FBXW) subfamily includes ten proteins including FBXW1 (also known as beta-transducin repeat-containing protein, β-TRCP1 or BTRC), FBXW2, FBXW4, FBXW5, FBXW7 (also known as FBW7, HCdc4, SEL-10, FBX30, and HAgo), FBXW8 (also known as FBXW6), FBXW9, FBXW10, FBXW11 (also known as β-TRCP2), and FBXW12 [8]. *FBXW3* is known as *FBXW4* Pseudogene 1 (*FBXW4P1*), thus does not belong to the *FBXW* subfamily.

FBXW7 is a tumor suppressor that acts through negative regulation of many oncogenic proteins, such as c-Myb, Mediator Complex Subunit 13 (MED13), Kruppel-like factor 2 (KLF2), KLF5, granulocyte colony stimulating factor receptor (G-CSFR), EYA Transcriptional Coactivator And Phosphatase 1 (EYA1), neurofibromatosis type 1, nuclear factor E2-related factor 1, p100/nuclear factor-κB2 (NF-κB2), GATA Binding Protein 3 (GATA3), JunB, Myeloid cell leukemia-1 (Mcl-1), c-Myc, Cyclin E, cyclin-dependent kinase 2 (CDK2), Hes Family BHLH Transcription Factor 1 (HES1), Cyclin D1, sterol regulatory element binding protein (SREBP), c-Jun, Hypoxia inducible factor-1α (HIF-1α), Notch1, DEK, Enolase 1 (ENO1), Yes-associated protein (YAP), mammalian target of rapamycin (mTOR), Ki-67, DNA Topoisomerase II Alpha (TOP2A), coiled-coil-domain containing 6 (CCDC6), Aurora kinase A (Aurora-A), Notch4, proliferation cell nuclear antigen (PCNA), and MYCN (see details in review [9]). These FBXW7 substrate proteins function as cell-cycle promoters or oncogenic regulators of cell proliferation, growth, and apoptosis. Loss or downregulated expression of FBXW7 leads to accumulation of these substrates, resulting in promotion of oncogenesis. In contrast, FBXW5 has been found to ubiquitinate Deleted in Liver Cancer 1 (DLC1), a tumor suppressor, thus acting as an oncogene to promote non-small cell lung cancer cell growth [10].

Ubiquitination of the substrate proteins leads to proteasome-mediated degradation, which needs the concerted action of ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s) [11]. The E2s control ubiquitin chain assembly and the E3s decide the substrate specificity. FBXW7 is an E3 ligase that binds to its substrates through the WD repeat domain, while it binds to Skp1 via the F-box motif. Skp1 binds to CUL1 and CUL1 binds to RBX1 that binds to and recruits the E2. The 3rd and 4th repeat domains of the WD40 of FBXW7 contain highly conserved arginine residues (R465, R479, and R505) [12], which bind with high affinity to Cdc4 phosphodegron (CPD), a consensus phosphopeptide motif (S/T-P-x-x-S/E). CPD is the common phosphorylation motif of most FBXW7 substrates. When glutamate or phosphorylation offers a negative charge, the serine or threonine in the "+4" position of CPD can be phosphorylated. Glycogen synthase kinase 3 (GSK-3) phosphorylates many of the FBXW7 substrates such as HIF-1α and JunB [9].

The objective of the present study was to examine genetic alterations and mRNA expression of the *FBXW* family members in human prostate cancer through analyzing the publicly available

Table 1. Data sources

Datasets‡	Author	Year	Included cases (n=1303)	Excluded cases* (n=77)
MCTP	Grasso [18]	2012	59	NA (n=60)
SU2C/PCF Dream Team	Abida [19]	2019	427	Unknown (n=2)
Multi-Institute	Beltran [20]	2016	81	
Broad/Cornell	Barbieri [21]	2012	112	NA (n=11)
Fred Hutchinson CRC	Kumar [22]	2016	61	Not profiled (n=2)
SMMU	Ren [23]	2017	65	
TCGA	TCGA data		498	Not profiled (n=2)

‡MCTP, Michigan Center for Translational Pathology; SU2C/PCF Dream Team, Stand Up to Cancer/Prostate Cancer Foundation Dream Team; Fred Hutchinson CRC, Fred Hutchinson Cancer Research Center; SMMU, Second Military Medical University (Shanghai); TCGA, The Cancer Genome Atlas. *Cases were excluded from analysis if the sample types were "Not available (NA)", "Unknown", or "Not profiled"; the number of cases excluded is shown.

datasets. We found that *FBXW* genes had differential mRNA expression in prostate cancer and other cancer types and their gene amplifications were significantly more in mCRPCs than primary prostate cancers. *FBXW7* mRNA expression was consistently decreased in primary prostate cancers compared to normal prostate tissues, which is consistent to its role as a tumor suppressor.

Materials and methods

Data sources

The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/tcga) was a public databank managed by the National Cancer Institute, National Institutes of Health. The University of ALabama CANcer data-portal (UALCAN) [13] was used to inquire mRNA expression levels in human normal prostate tissues (n=52) and primary prostate cancers (n=497) archived at TCGA. A second data source from the Prostate Cancer Transcriptome Atlas (PCTA, www.thepcta.org) [14] was also used to analyze mRNA expression levels. PCTA archives the transcriptome data of 1321 clinical specimens from 38 prostate cancer cohorts. We filtered through the datasets and included 260 samples of mCRPCs, 1061 samples of primary prostate cancers, and 794 samples of benign prostate tissues.

cBioPortal for Cancer Genomics (www.cbioportal.org) [15, 16] was used for analysis of gene alterations. cBioPortal has archived 20 datasets for gene alterations in human prostate cancers. As described in our previous study [17], we filtered through the datasets and excluded the datasets that potentially used overlapping original samples according to the linked publications. Seven datasets were included, which did not appear to have overlapping original samples (**Table 1**, published previously [17]).

As described previously [17], the data of Michigan Center for Translational Pathology (MCTP) were generated by Dr. Arul Chinnaiyan's and Dr. Scott Tomlins' labs at the University of Michigan [18]; the data of Stand Up to Cancer/Prostate Cancer Foundation Dream Team (SU2C/PCF Dream Team) were generated by researchers at Dana-Farber Cancer Institute, Karmanos Cancer Institute, Memorial Sloan Kettering Cancer Center, Royal Marsden, University of Michigan, University of Washington, and Weill Cornell Medicine [19]; the data of Multi-Institute were generated by researchers at Weill Cornell Medicine [20]; the data of Broad/Cornell were generated by Dr. Levi Garraway's lab at Broad Institute and Dr. Mark Rubin's lab at Weill Cornell Medicine [21]; the data of Fred Hutchinson Cancer Research Center were generated by researchers at the University of Washington and Fred Hutchinson Cancer Research Center [22]; the data of the Second Military Medical University (Shanghai) (SMMU) were generated by researchers at Shanghai Changhai Hospital and Fudan University Cancer Center [23]. A total of 1303 cases of prostate cancer samples (n=1303) were included, while 77 cases were excluded from analysis because the sample types were "Not available (NA)", "Unknown", or "Not profiled" (**Table 1**).

Bioinformatics analysis

UALCAN portal (http://ualcan.path.uab.edu/cgi-bin/ualcan-res.pl) was used to inquire

mRNA expression levels according to the online instructions. The focus was on *FBXW7* and other *FBXW* family members. The target gene identification (ID) (e.g., *FBXW7*) was typed in the scan box and the TCGA dataset (e.g., Prostate adenocarcinoma) was chosen. Then, mRNA expression, survival (with tumors categorized into low and high expression groups), promoter methylation, and pan-cancer view were explored. Besides comparison between normal prostate tissues and primary prostate cancers, prostate cancer patients' race, age, Gleason score (GS), molecular signature, lymph node metastasis status, and tumor suppressor 53 (*TP53*) mutation status were also analyzed in relationship to mRNA expression levels using UALCAN.

We also used PCTA query tools to analyze gene expression levels of 10 *FBXW* family members. The bioinformatics analysis procedures are briefly described here: first, we chose PCTA dataset and typed gene ID (e.g., *FBXW10*) in the query box above "Run Expression View" button on the main page of PCTA; second, we chose "By Disease Course" on the next page and clicked "Analysis start"; then, we reached the result page. It showed that the fold change (Log_2 value) was 0.135 and *P*-value was 0.147 when Rank-Sum Test was performed between mCRPCs and primary prostate cancers; and the fold change was 0.054 and *P*-value was less than 0.001 when Rank-Sum Test was performed between primary prostate cancers and benign prostate tissues.

We used TCGA query tools to find gene copy number alterations and simple somatic mutations of 10 *FBXW* genes. TCGA has archived 2326 cases of human prostate cancers with information of gene alterations. We included 487 cases of primary prostate cancers and 116 cases of normal prostate tissues in analysis of gene copy number gains and gene copy number losses. We included 498 cases of primary prostate cancers and 118 cases of normal prostate tissues in analysis of gene simple somatic mutations. The cases were chosen based on what were available in the search tools of TCGA. The bioinformatics analysis procedures are briefly described here: first, we typed "TCGA" and chose "GDC Data Portal" (Genomic Data Commons Data Portal); second, we clicked "Prostate"; third, we chose "TCGA" in "Program" and "primary tumor" in "Sample Type"; fourth, we chose "Genes (20202)" and then reached the result page where showed the gene copy number alterations (gains and losses) and simple somatic mutations.

We used cBioPortal query tools to find genetic alterations of 10 *FBXW* genes as previously described [17]. The bioinformatics analysis procedures are briefly described here: first, we chose "Prostate" organ type on the main page of cBioPortal; second, we chose the dataset named "Metastatic Prostate Adenocarcinoma (MCTP, Nature 2012)" and clicked the round button on the right side; third, we typed in gene names (e.g., *FBXW8*) in the query box on right top corner of the page; fourth, we clicked "Charts" below the query box and clicked "Deselect all"; fifth, we chose "Sample Type" and we could see a pie chart on this page where we chose the portion named "metastatic" and clicked "Query" on the right side of query box; sixth, we moved the mouse on the "4%" on the right side of "*FBXW8*", which showed "altered/profiled 2/48". That means the number of overall *FBXW8* gene alterations in metastatic prostate cancers was 2, and the number of total cases was 48. We used Object Query Language (OQL) to do queries of the 10 *FBXW* genes. The gene alterations were categorized into copy number alterations (amplifications and deep deletions) and mutations (missense mutations and truncating mutations) according to cBioPortal. Prostate cancer sample types were categorized into primary prostate cancers (n=742), and metastatic prostate cancers (n=584, of which 583 cases were mCRPCs). We identified and calculated the numbers and percentages of overall gene alterations and individual categories of gene alterations after pooling the query results from the 7 datasets.

Statistical analysis

R software package [R version 3.5.2 (2018-12-20), R Core Team (2018); R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/] was used to perform Fisher's exact test between two sample types. $P<0.05$ was considered statistically significant. UALCAN's statistical analysis was described previously [13].

Table 2. mRNA expression levels in human normal prostate tissues (n=52) and primary prostate cancers (n=497) based on analysis of TCGA data using UALCAN data portal

Gene name	Gene ID	Expression in Normal Prostate median (range)§	Expression in Prostate Cancer median (range)§	*P*-value
FBXW1	*BTRC*	11.0 (4.9-17.0)	11.4 (2.8-22.7)	3.576800E-01
FBXW2	*FBXW2*	12.7 (5.6-21.6)	13.5 (2.8-24.5)	2.797000E-01
*FBXW4**	*FBXW4*	65.0 (25.3-91.6)	71.9 (34.4-110.2)	2.985400E-04
FBXW5	*FBXW5*	97.8 (46.5-148.4)	104.3 (45.2-169.5)	3.440000E-04
FBXW7	*FBXW7*	6.7 (3.4-11.9)	5.7 (2.1-9.5)	3.183100E-06
FBXW8	*FBXW8*	6.8 (3.2-11.9)	7.9 (1.9-15.3)	1.918290E-03
FBXW9	*FBXW9*	11.2 (4.1-20.1)	17.1 (4.5-30.7)	1.624478E-12
FBXW10	*FBXW10*	0.02 (0-0.07)	0.03 (0-0.12)	1.631409E-05
FBXW11	*FBXW11*	21.7 (8.8-46.2)	21.7 (2.6-45.0)	1.669990E-01
FBXW12	*FBXW12*	0 (0-0.07)	0 (0-0.05)	2.466000E-01

§unit, transcript per million. *Red indicates significantly higher mRNA levels in primary prostate cancers than normal prostate tissues; green indicates significantly higher mRNA levels in normal prostate tissues than primary prostate cancers. BTRC, beta-transducin repeat containing; FBXW, F-box/WD40 repeat-containing protein; TCGA, The Cancer Genome Atlas; UALCAN, University of ALabama CANcer data-portal.

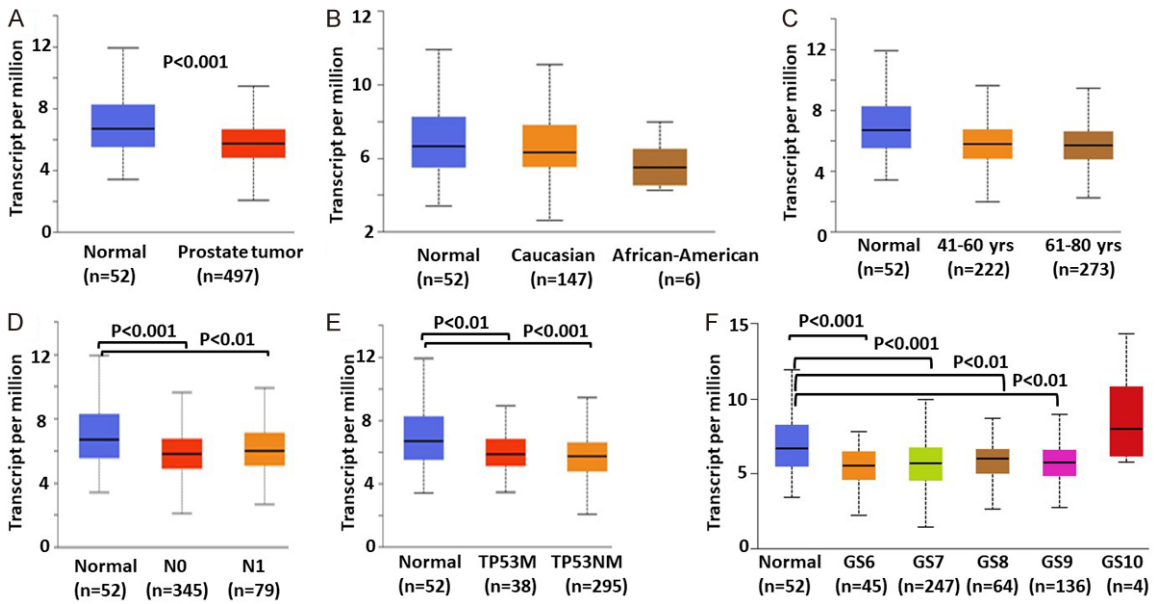

Figure 1. *FBXW7* mRNA expression levels in human primary prostate cancers and normal prostate tissues. TCGA data were analyzed with UALCAN tools. A. *FBXW7* mRNA expression levels in human primary prostate cancers and normal prostate tissues. B. Prostate cancer patients' race was assigned as Caucasians and African-Americans. C. Prostate cancer patients were divided into young (41-60 years old) and old (61-80 years old) groups. D. Prostate cancer patients were assigned as without (N0) and with (N1) lymph node metastases. E. Prostate cancers were assigned as with *TP53* mutations (*TP53M*) and without *TP53* mutations (*TP53NM*). F. Prostate cancers were assigned into different groups based on Gleason score (GS).

Results

FBXW7 mRNA expression was decreased in human primary prostate cancer

Using UALCAN tools to analyze TCGA data, we found that *FBXW7* mRNA expression levels were significantly decreased in human primary prostate cancers compared to normal prostate tissues (**Table 2** and **Figure 1A**). In contrast, mRNA expression levels of *FBXW4, 5, 8, 9* and *10* were significantly increased in human primary prostate cancers compared to normal prostate tissues (**Table 2**). The mRNA expres-

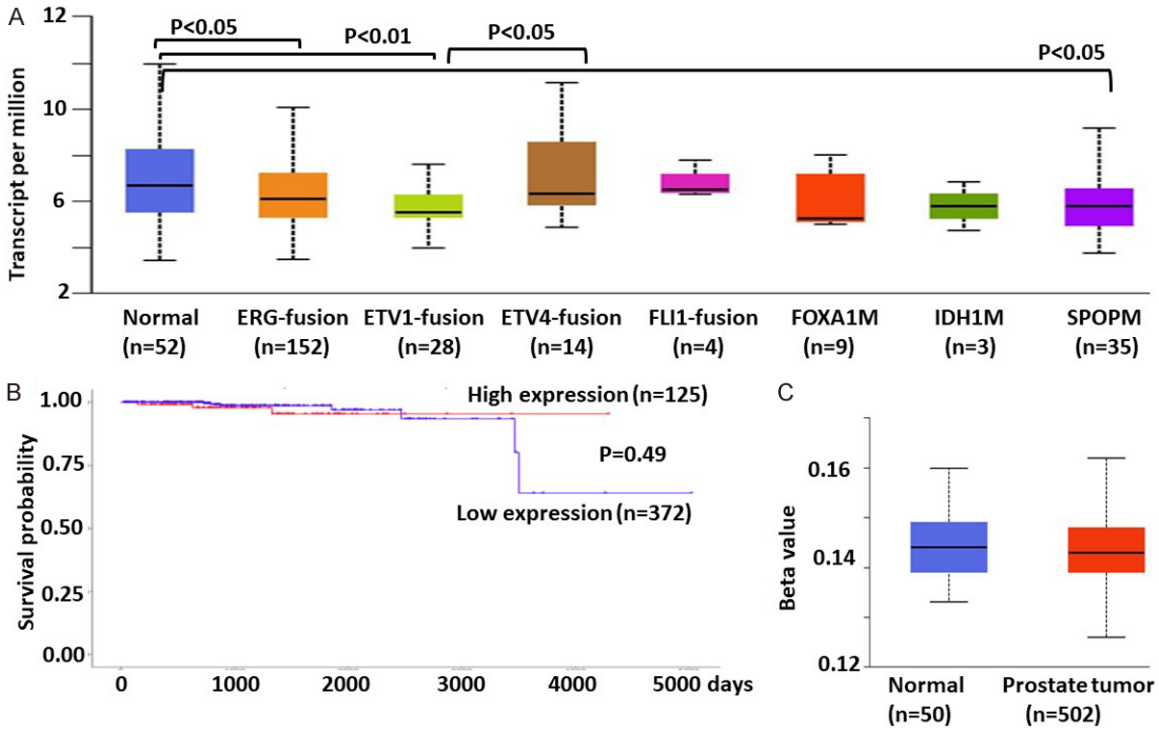

Figure 2. Relationship of *FBXW7* mRNA expression to prostate cancers' molecular signatures and patients' survival. TCGA data were analyzed with UALCAN tools. A. Prostate cancers were assigned into different molecular signature groups; *FOXA1M*, *FOXA1* mutation; *IDH1M, IDH1* mutation; *SPOPM, SPOP* mutation. B. Prostate cancer patients were assigned into high *FBXW7* mRNA expression and low *FBXW7* mRNA expression groups. C. *FBXW7* gene promoter methylation levels were represented with Beta value that indicates levels of DNA methylation ranging from 0 (unmethylated) to 1 (fully methylated); different Beta value cut-off has been considered to indicate hyper-methylation [Beta value: 0.7-0.5] or hypo-methylation [Beta-value: 0.3-0.25] [1, 2].

Table 3. mRNA expression levels in human primary prostate cancers (n=1061) compared to benign prostate tissues (n=794) based on the Prostate Cancer Transcriptome Atlas (PCTA) data

Gene ID	Fold change§	*P*-value (Rank-Sum Test)
*BTRC**	0.024	0.025
FBXW2	0.083	0.013
FBXW4	-0.02	0.002
FBXW5	-0.079	<0.001
FBXW7	-0.1	<0.001
FBXW8	0.087	<0.001
FBXW9	0.113	<0.001
FBXW10	0.054	<0.001
FBXW11	0.008	0.385
FBXW12	-0.041	0.052

*Red indicates significantly higher gene expression in primary prostate cancers than benign prostate tissues; green indicates significantly higher gene expression in benign prostate tissues than primary prostate cancers. §Log_2 values using benign prostate tissues as baseline.

sion levels of *FBXW1, 2, 11,* and *12* showed no significant differences between primary prostate cancers and normal prostate tissues (**Table 2**).

Further analysis showed that *FBXW7* mRNA levels had no significant differences between the Caucasians and African-Americans (**Figure 1B**), between the young (41-60 years old) and old (61-80 years old) patients (**Figure 1C**), between the tumors with or without lymph node metastases (**Figure 1D**), between the tumors with or without *TP53* mutations (**Figure 1E**), or among the tumors with different Gleason scores (**Figure 1F**). Although Gleason score 10 tumors showed increased *FBXW7* mRNA levels, the difference had no statistical significance due possibly to the small sample size (n=4).

Prostate cancer presents gene fusions such as transmembrane protease, serine 2 (*TMPRSS2*) fusion with erythroblast transformation-specif-

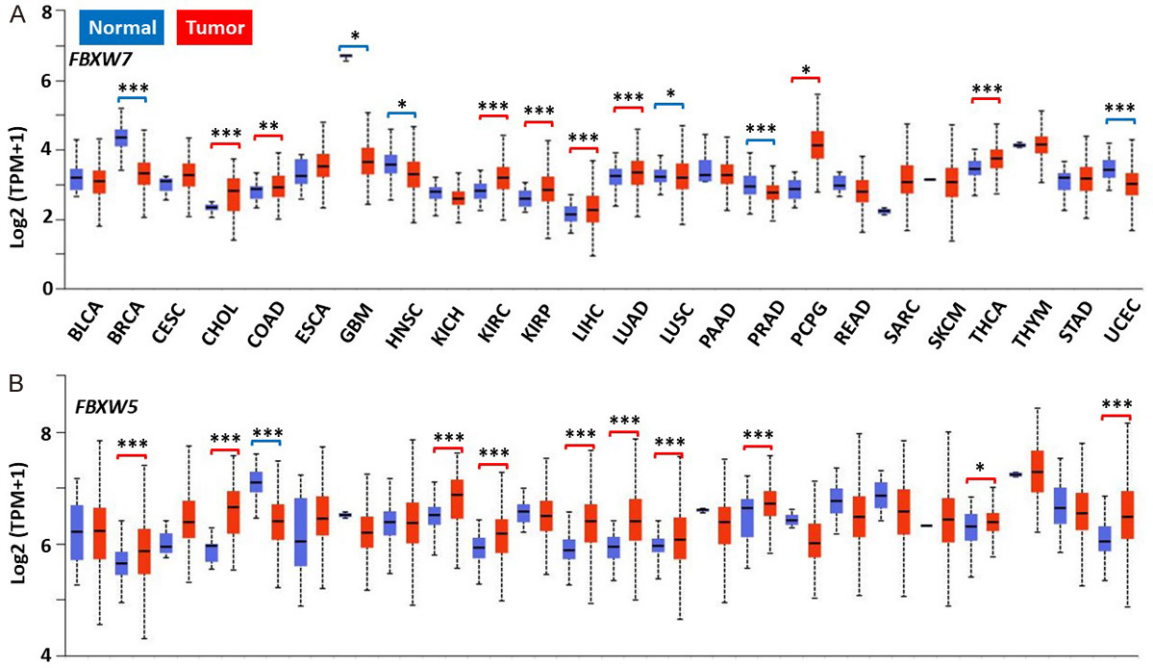

Figure 3. Pan-cancer view of *FBXW7* and *FBXW5* mRNA expression in 24 types of cancers and their corresponding normal tissues. TPM, transcript per million; *P<0.05, **P<0.01, and ***P<0.001. BLCA, Bladder urothelial carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and neck squamous cell carcinoma; KICH, Kidney chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; PAAD, Pancreatic adenocarcinoma; PRAD, Prostate adenocarcinoma; PCPG, Pheochromocytoma and paraganglioma; READ, Rectal adenocarcinoma; SARC, Sarcoma; SKCM, Skin cutaneous melanoma; THCA, Thyroid carcinoma; THYM, Thymoma; STAD, Stomach adenocarcinoma; UCEC, Uterine corpus endometrial carcinoma.

ic (*ETS*)-related gene (*ERG*) (*TMPRSS2-ERG* fusion), *TMPRSS2-ETS* Variant Transcription Factor 1 (*ETV1*) fusion, and *TMPRSS2-ETV4* fusion [24], as well as Solute Carrier Family 45 Member 3 (*SLC45A3*)-Friend Leukemia Virus Integration 1 (*FLI1*) fusion [25]. Gene mutations have also been found in prostate cancer, such as Forkhead Box A1 (*FOXA1*) [26], Isocitrate Dehydrogenase (NADP(+)) 1 (*IDH1*) [27], and Speckle Type BTB/POZ Protein (*SPOP*) [28]. We found that there were no significant differences in *FBXW7* mRNA expression levels among the prostate cancers with these molecular signatures, except between *ETV1*-fusion and *ETV4*-fusion (**Figure 2A**). Further, the patients with high levels of *FBXW7* mRNA expression appeared to have a better survival probability than the patients with low levels of *FBXW7* mRNA expression, but the difference was not statistically significant (P=0.49) (**Figure 2B**).

To corroborate the findings from analysis of TCGA data, we analyzed the PCTA data and found that *FBXW7* mRNA expression levels were decreased in primary prostate cancers compared to benign prostate tissues in this dataset (**Table 3**). mRNA expression levels of *FBXW8, 9*, and *10* were increased in primary prostate cancers, which was consistent with the TCGA dataset (**Table 3**). However, mRNA expression levels of *FBXW4* and *5* were increased in primary prostate cancers in the PCTA dataset (**Table 3**), which was inconsistent with the TCGA dataset.

FBXW7 and FBXW5 presented different patterns of mRNA expression among 24 cancer types

To explore *FBXW7* mRNA expression in other cancer types, we used the pan-cancer view function of UALCAN. Compared to the normal tissue of each cancer's organ of origin, *FBXW7* mRNA expression was significantly decreased in breast invasive carcinoma (BRCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), prostate adenocarcino-

Table 4. Gene copy number gains in primary prostate cancers vs normal prostate tissues

Gene ID	Primary prostate cancers (n=487)		Normal prostate tissues (n=116)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
BTRC	4	0.82%	1	0.86%	1.00E+00
FBXW2	9	1.85%	1	0.86%	6.96E-01
FBXW4	4	0.82%	1	0.86%	1.00E+00
FBXW5	8	1.64%	1	0.86%	1.00E+00
FBXW7	6	1.23%	1	0.86%	1.00E+00
FBXW8	12	2.46%	1	0.86%	4.80E-01
FBXW9	2	0.41%	0	0.00%	1.00E+00
FBXW10	13	2.67%	3	2.59%	1.00E+00
FBXW11	11	2.26%	5	4.31%	2.08E-01
FBXW12	1	0.21%	1	0.86%	3.48E-01

Table 5. Gene copy number losses in primary prostate cancers vs normal prostate tissues

Gene ID	Primary prostate cancers (n=487)		Normal prostate tissues (n=116)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
BTRC	36	7.39%	8	6.90%	1.00E+00
FBXW2	4	0.82%	1	0.86%	1.00E+00
FBXW4	36	7.39%	8	6.90%	1.00E+00
FBXW5	0	0.00%	0	0.00%	1.00E+00
FBXW7	10	2.05%	3	2.59%	7.23E-01
FBXW8	11	2.26%	1	0.86%	4.78E-01
FBXW9	9	1.85%	2	1.72%	1.00E+00
FBXW10	5	1.03%	2	1.72%	6.25E-01
FBXW11	1	0.21%	0	0.00%	1.00E+00
FBXW12	12	2.46%	1	0.86%	4.80E-01

Table 6. Gene simple somatic mutations in primary prostate cancers vs normal prostate tissues

Gene ID	Primary prostate cancers (n=498)		Normal prostate tissues (n=118)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
BTRC	1	0.20%	0	0.00%	1.00E+00
FBXW2	0	0.00%	0	0.00%	1.00E+00
FBXW4	1	0.20%	0	0.00%	1.00E+00
FBXW5	2	0.40%	1	0.85%	4.72E-01
FBXW7	1	0.20%	0	0.00%	1.00E+00
FBXW8	1	0.20%	0	0.00%	1.00E+00
FBXW9	1	0.20%	1	0.85%	3.47E-01
FBXW10	3	0.60%	0	0.00%	1.00E+00
FBXW11	3	0.60%	1	0.85%	5.74E-01
FBXW12	1	0.20%	0	0.00%	1.00E+00

ma (PRAD), and uterine corpus endometrial carcinoma (UCEC) (**Figure 3A**). In contrast, *FBXW7* mRNA expression was significantly increased in cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), pheochromocytoma and paraganglioma (PCPG), and thyroid carcinoma (THCA) (**Figure 3A**). Compared to normal tissues, *FBXW5* mRNA expression was significantly increased in breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) (**Figure 3B**). *FBXW5* mRNA expression was only significantly decreased in colon adenocarcinoma (COAD) (**Figure 3B**).

The mechanisms of differential FBXW gene expression were not clear

To understand the causes of the differential expression of *FBXW* family, we analyzed the TCGA dataset and found that primary prostate cancers and normal prostate tissues presented rare gene copy number gains (**Table 4**), losses (**Table 5**), and simple somatic mutations (**Table 6**). There were no significant differences between primary prostate cancers and normal prostate tissues (**Tables 4**-**6**). *FBXW7* promoter methylation levels did not differ significantly between primary prostate cancers and normal prostate tissues (**Figure 2C**).

FBXW genes were differentially expressed between mCRPCs and primary prostate cancers

Analyzing the PCTA data, we found that mRNA expression levels of

Table 7. mRNA expression levels in mCRPCs (n=260) compared to primary prostate cancers (n=1061) based on the PCTA data

Gene ID	Fold change[§]	*P*-value (Rank-Sum Test)
BTRC	-0.242	<0.001
FBXW2	-0.005	0.709
FBXW4	-0.106	0.003
*FBXW5**	0.167	<0.001
FBXW7	0.128	<0.001
FBXW8	0.124	<0.001
FBXW9	0.065	0.011
FBXW10	0.135	0.147
FBXW11	0.005	0.961
FBXW12	0.037	0.002

*Red indicates significantly higher gene expression in metastatic castration-resistant prostate cancers (mCRPCs) than primary prostate cancers; green indicates significantly higher gene expression in primary prostate cancers than mCRPCs. [§]Log_2 values using primary prostate cancers as baseline.

Table 8. Overall gene alterations in metastatic vs primary prostate cancers

Gene ID	Metastatic (n=584)		Primary (n=742)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
*BTRC**	33	5.65%	19	2.56%	4.34E-03
FBXW2	37	6.34%	14	1.89%	3.73E-05
FBXW4	31	5.31%	17	2.29%	4.51E-03
FBXW5	48	8.22%	10	1.35%	8.83E-10
FBXW7	36	6.16%	5	0.67%	5.20E-09
FBXW8	52	8.90%	11	1.48%	2.84E-10
FBXW9	29	4.97%	9	1.21%	8.15E-05
FBXW10	36	6.16%	11	1.48%	7.10E-06
FBXW11	33	5.65%	14	1.89%	2.71E-04
FBXW12	26	4.45%	10	1.35%	9.33E-04

*Red indicates significantly more gene alterations in metastatic than primary prostate cancers.

FBXW5, 7, 8, 9, and *12* were significantly higher in mCRPCs than primary prostate cancers, whereas mRNA expression levels of *FBXW1* and *4* were significantly lower in mCRPCs than primary prostate cancers (**Table 7**). To reveal the genetic changes underlying the differential mRNA expression, we analyzed 7 datasets archived at cBioPortal. We found that all 10 *FBXW* genes had significantly more overall gene alterations in mCRPCs than primary prostate cancers (**Table 8**). Further analysis showed that all 10 *FBXW* genes had significantly more gene amplifications in mCRPCs than primary prostate cancers (**Table 9**). *FBXW5* and *7* had significantly more gene deep deletions in mCRPCs than primary prostate cancers (**Table 10**). *FBXW7* had significantly more gene missense mutations in mCRPCs than primary prostate cancers (**Table 11**). There were not any significant differences in gene truncating mutations between mCRPCs and primary prostate cancers (**Table 12**).

Discussion

TCGA is a very rich databank that has been very useful in performing large scale analyses of the entire genome in most cancers [29]. UALCAN is a free portal for high-throughput analysis of gene mRNA expression [13]. The PCTA is also a very useful source for transcriptome analysis [14]. In the present study, we analyzed these publicly available datasets and found that *FBXW7* mRNA expression was significantly decreased in primary prostate cancers compared to normal prostate tissues, whereas mRNA expression of *FBXW8-10* was significantly increased in primary prostate cancers compared to normal prostate tissues. These findings were consistent between TCGA and PCTA datasets. We noted that mRNA expression levels of *FBXW1, 2, 4,* and *5* were inconsistent between TCGA and PCTA datasets (**Tables 2**, **3**). We speculate that this could be caused by different samples and different measurements used in the two datasets.

One interesting observation is the contrast between *FBXW7* and *FBXW5*. *FBXW7* is a potent tumor suppressor because most of its target substrates are potential growth promoters [30]. A study based on the Catalogue of Somatic Mutations in Cancer (COSMIC) database found that *FBXW7* gene has the largest mutation frequency among 10 *FBXW* family members [12]. *FBXW7* mRNA expression was significantly decreased in breast invasive carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, and

Table 9. Gene amplifications in metastatic vs primary prostate cancers

Gene ID	Metastatic (n=584)		Primary (n=742)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
*BTRC**	14	2.40%	2	0.27%	4.88E-04
FBXW2	32	5.48%	12	1.62%	1.45E-04
FBXW4	14	2.40%	2	0.27%	4.88E-04
FBXW5	39	6.68%	10	1.35%	4.38E-07
FBXW7	23	3.94%	3	0.40%	2.88E-06
FBXW8	49	8.39%	8	1.08%	2.81E-11
FBXW9	24	4.11%	3	0.40%	1.41E-06
FBXW10	28	4.79%	7	0.94%	1.61E-05
FBXW11	28	4.79%	10	1.35%	2.08E-04
FBXW12	22	3.77%	5	0.67%	9.86E-05

*Red indicates significantly more gene amplifications in metastatic than primary prostate cancers.

Table 10. Gene deep deletions in metastatic vs primary prostate cancers

Gene ID	Metastatic (n=584)		Primary (n=742)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
BTRC	17	2.91%	15	2.02%	3.68E-01
FBXW2	1	0.17%	2	0.27%	1.00E+00
FBXW4	17	2.91%	15	2.02%	3.68E-01
*FBXW5**	6	1.03%	0	0.00%	7.19E-03
FBXW7	8	1.37%	2	0.27%	2.62E-02
FBXW8	2	0.34%	2	0.27%	1.00E+00
FBXW9	4	0.68%	4	0.54%	7.37E-01
FBXW10	5	0.86%	2	0.27%	2.51E-01
FBXW11	4	0.68%	1	0.13%	1.76E-01
FBXW12	4	0.68%	4	0.54%	7.37E-01

*Red indicates significantly more gene deep deletions in metastatic than primary prostate cancers.

uterine corpus endometrial carcinoma, while its expression was significantly increased in cholangiocarcinoma, colon adenocarcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, pheochromocytoma and paraganglioma, and thyroid carcinoma. In contrast to *FBXW7*, *FBXW5* encodes a protein that has an oncogenic potential, which has been shown to have a growth-promoting effect in non-small cell lung cancer [10] and an oncogenic role in gastric cancer tumorigenesis and metastasis [31]. *FBXW5* mRNA expression was significantly increased in breast invasive carcinoma, cholangiocarcinoma, kidney chromophobe, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, thyroid carcinoma, and uterine corpus endometrial carcinoma, whereas its expression was only significantly decreased in colon adenocarcinoma. These two genes exhibited opposite patterns of mRNA expression in breast invasive carcinoma, colon adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, and uterine corpus endometrial carcinoma (**Figure 3**). Functionally, it has been reported that *FBXW7* is a tumor suppressor [9], whereas *FBXW5* may be an oncogene [10]. It is not clear how the *FBXW* genes are differentially expressed as we found that there were not any significant differences in gene copy number gains, losses, or gene simple somatic mutations between primary prostate cancers and normal prostate tissues. *FBXW7* gene promoter methylation levels were not different between primary prostate cancers and normal prostate tissues, which rules out promoter methylation as a factor.

Another observation worth mentioning is that mRNA expression levels of *FBXW5, 7, 8, 9*, and *12* were significantly higher in mCRPCs than primary prostate cancers. This could be explained by higher rates of gene amplifications. However, mRNA expression levels of *FBXW1* and *4* were significantly lower in mCRPCs than primary prostate cancers, though increased gene amplifications were also found. This makes the explanation difficult. Furthermore, it is not known if the protein expression levels are consistent with the mRNA expression levels, which should be addressed in the future studies.

In summary, our findings suggest that different *FBXW* genes have differential mRNA expression in prostate cancer and other cancer types and their gene amplifications are significantly more in mCRPCs than primary prostate can-

Table 11. Gene missense mutations in metastatic vs primary prostate cancers

Gene ID	Metastatic (n=584)		Primary (n=742)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
BTRC	2	0.34%	2	0.27%	1.00E+00
FBXW2	3	0.51%	0	0.00%	8.52E-02
FBXW4	0	0.00%	0	0.00%	1.00E+00
FBXW5	3	0.51%	0	0.00%	8.52E-02
*FBXW7**	6	1.03%	0	0.00%	7.19E-03
FBXW8	1	0.17%	1	0.13%	1.00E+00
FBXW9	0	0.00%	1	0.13%	1.00E+00
FBXW10	3	0.51%	2	0.27%	6.60E-01
FBXW11	1	0.17%	3	0.40%	6.35E-01
FBXW12	0	0.00%	1	0.13%	1.00E+00

*Red indicates significantly more gene missense mutations in metastatic than primary prostate cancers.

Table 12. Gene truncating mutations in metastatic vs primary prostate cancers

Gene ID	Metastatic (n=584)		Primary (n=742)		*P*-value (Fisher's Exact Test)
	Number	Percent	Number	Percent	
BTRC	0	0.00%	1	0.13%	1.00E+00
FBXW2	1	0.17%	0	0.00%	4.40E-01
FBXW4	0	0.00%	0	0.00%	1.00E+00
FBXW5	0	0.00%	0	0.00%	1.00E+00
FBXW7	0	0.00%	0	0.00%	1.00E+00
FBXW8	0	0.00%	0	0.00%	1.00E+00
FBXW9	1	0.17%	1	0.13%	1.00E+00
FBXW10	0	0.00%	0	0.00%	1.00E+00
FBXW11	0	0.00%	0	0.00%	1.00E+00
FBXW12	0	0.00%	0	0.00%	1.00E+00

cers. *FBXW7* mRNA expression is consistently decreased in primary prostate cancers compared to normal prostate tissues. Future studies shall explore FBXW7 protein expression in prostate cancer and determine its role in prostate cancer initiation and progression.

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Disclosure of conflict of interest

None.

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