Article

Portrait of Molecular Signaling and Putative Therapeutic Targets in Prostate Cancer with ETV4 Fusion

Ye Ji Shin 1, Jae Won Yun 2,* and Hong Sook Kim 1,*

1 Department of Biological Sciences, Sungkyunkwan University, Suwon 19419, Korea
2 Veterans Medical Research Institute, Veterans Health Service Medical Center, Seoul 05368, Korea
* Correspondence: jwyunmd@gmail.com (J.W.Y.); hong2kim2@gmail.com (H.S.K.)

Abstract: Gene fusion between androgen receptor (AR) response genes and E26 transformation-specific (ETS) family members increases the gene expression of ETS family members, and promotes tumorigenesis in prostate cancer. However, the molecular features of ETV4 fusion in prostate cancer are not fully understood, and drugs targeting ETV4 fusion have not been developed. To examine key cellular signaling pathways and explore therapeutic targets and drugs for ETV4-fusion-positive prostate cancer, we analyzed RNA sequencing data and clinical information for prostate cancer. The ETV4-fusion-positive group was selected through prior study and analysis comparing ETV4-fusion-positive and -negative groups was conducted using a Pearson correlation test. We obtained 393 genes correlated with ETV4 expression. Pathway analysis was performed using over-representation analysis (ORA), and six cancer-specific molecular signaling pathways (the irinotecan pathway, metabolism, androgen receptor signaling, interferon signaling, MAPK/NF-κB signaling, and the tamoxifen pathway) were altered in the ETV4-fusion-positive group. Furthermore, a gene–drug database was used to find an actionable drug and therapeutic target for the ETV4-fusion-positive group. Here, we have identified significantly altered genes and oncogenic signaling pathways in ETV4-fusion-positive prostate cancer, and we suggest therapeutic targets and potential drugs for ETV4-fusion-positive prostate patients.

Keywords: cellular signaling pathways; drug repurposing; ETV4; prostate cancer

1. Introduction

Prostate cancer is the second-most frequent cancer type in males following lung cancer, and it ranked as the fifth-leading cause of death in males in 2020 [1]. It is also the most commonly diagnosed type of cancer in developed countries, such as those in North America and Northern/Western Europe.

Prostate cancer frequently harbors the chromosomal rearrangement of the E26 transformation-specific (ETS) gene, and the most frequent fusion partner gene is TMPRSS2. ERG and TMPRSS2 fusion is found in about 50% of prostate cancers [2], and ETV1 and TMPRSS2 fusion is found in 5% of prostate cancers. Fusion of the ETS gene results in the high expression of ERG or ETV1, and it is an important cause of prostate tumorigenesis and tumor development [3,4]. In addition to its oncogenic effects, the clinical relevance of ETS gene fusion has been well described [4,5]. Since ETS gene fusion was first discovered, novel 5′ and 3′ partner genes, such as ETV4, ETV5, and SKIL, have been discovered in studies [5]. The structural variation of ETS variant transcription factor 4 (ETV4), defined as another driver mutation, was observed in 2% of prostate cancers [6]. TMPRSS2 is a major fusion companion of ETV4, and a significant portion fuses by gene-intergenic fusion, an unusual mechanism which produces chimeric RNA [7]. Other genes, such as STAT3 and PMEPA1, are also known to fuse with ETV4. Also, there are some cases where the mechanism by which ETV4 is highly expressed in prostate cancer is not well-defined. The overexpression
of ETV4 promotes cell proliferation by upregulating cancer-related genes and the epithelial–mesenchymal transition (EMT) by activating EMT-specific transcription factors [8]. ETV4 increases metastasis by activating PI3K kinase-RAS signaling in mouse prostate cancer models [9]. Overall, the oncogenic effects of ETV4 are well-described in both in vivo and in vitro models [8]. However, molecular features, such as gene expression and signaling pathways, in ETV4-fusion-positive prostate cancers have not been fully elucidated. Indeed, therapeutic targets and candidate targeted drugs for ETV4-overexpressing prostate cancer remain unknown. Thus, we here systematically analyzed RNA sequencing (RNA-seq) data obtained from The Cancer Genome Atlas (TCGA) database and identified oncogenic signaling pathways activated in ETV4-fusion-positive prostate cancer, which results in ETV4 overexpression. We further performed gene–drug network analysis and investigated therapeutic targets and candidate drugs in ETV4-fusion-positive prostate cancer.

2. Materials and Methods

2.1. Selection of Case and Control Groups for Analysis

Structural variation, mutation information, and gene expressions from The Cancer Genome Atlas (TCGA) prostate cancer molecular taxonomy study was used to clarify subtypes of prostate cancer [10]. A total of 14 ETV4-fusion-positive samples were selected as the case group, and 86 samples without any driver mutations, such as ERG fusion, ETV1 fusion, SPOP mutation, FOXA1 mutation, or IDH1 mutation, were selected as the control group.

2.2. Selection of Genes Associated with ETV4 Expression

RNA expression data with upper quantile normalization was obtained from the TCGA Prostate Adenocarcinoma (PRAD) study from the Broad GDAC Firehose website (http://gdac.broadinstitute.org, accessed on 12 December 2021). Sample type code 01, indicating primary solid tumor samples, and sample type code 11, indicating adjacent normal tissue, were used in this study. ETV4 fusion with androgen-responsive genes results in ETV4 overexpression in prostate cancers [6]; thus, we selected genes associated with ETV4 expression via the Pearson correlation test using an absolute value of Pearson correlation coefficient over 0.3 (|R| > 0.3), assuming that the expression fluctuation of downstream genes affected by ETV4 would be proportional to the expression of ETV4.

The CancerMine database (http://bionlp.bcgsc.ca/cancermine, accessed on 12 December 2021) was used to identify cancer-related genes [11]. This database contains genes and their functional effects, such as tumor suppressor, oncogene, and driver, with cancer types.

2.3. Pathway Analysis

The genes significantly correlated with ETV4 expression in prostate cancer were used to perform over-representation analysis (ORA) via ConsensusPathDB (CPDB, http://cpdb.molgen.mpg.de, accessed on 22 June 2021) with the options of allowing a minimum of two genes to overlap from the input list and a p-value < 0.01 cutoff. Biocarta, Ehmnn, Humancyc, Inoh, Kegg, Manual upload, Netpath, Pharmgkb, Pid, Reactome, Signalink, Smpdb, and Wikipathways, which were curated and provided by CPDB, were used for pathway analysis.

2.4. Therapeutic Targets and Candidate Drugs

A drug-target database containing target genes, variants, disease, and drugs was obtained from the Clinical Interpretation of Variants in Cancer platform (CIViC, https://civicdb.org/home, accessed on 22 June 2021). Gene–drug network analysis was performed using genes involved in cancer-specific pathways of ETV4-fusion-positive prostate cancer, and therapeutic targets and potential actionable drugs were obtained via the network analysis.
2.5. Statistical Analysis and Visualization

Clinical characteristics, such as age, prostate-specific antigen (PSA) level, Gleason score, tumor stage (T stage and M stage), laterality, and ethnicity were analyzed between the ETV4-fusion-positive and -negative groups to check differences in clinical information via the moonBook package (https://cran.r-project.org/web/packages/moonBook/, accessed on 7 October 2021) in software R version 3.6.3. Because the clinical information provided from TCGA is limited, only the available data sets were used for this statistical analysis.

Genes significantly correlated with ETV4 expression were identified via a Pearson correlation test using the cor.test function in R. Cancer-specific cellular signaling pathways and involved genes altered in ETV4-fusion-positive prostate cancer were visualized at the gene level using an R package named ComplexHeatmap [12]. Graphs, except for heatmaps, were visualized using the R package ggplot2, and statistical significance was calculated and labeled by the R package ggpubr. The gene–drug network and gene-pathway network were visualized via Cytoscape version 3.8.2 [13].

3. Results

3.1. Comparison of Patient Characteristics

ETV4-fusion-positive prostate cancers were selected based on structural variation data and gene expression data according to a previous report [10]. Patient characteristics between 14 ETV4-fusion-positive prostate cancers and 86 ETV4-fusion-negative prostate cancers were analyzed. Age, PSA level, Gleason score, tumor stage (T stage and M stage), laterality, and ethnicity were compared between the ETV4-fusion-positive and -negative prostate cancer groups, and no significant difference was observed (Table 1).

Table 1. Comparison of patient clinical characteristics of prostate cancer groups.

|                      | ETV4-Fusion-Positive (n = 14) | ETV4-Fusion-Negative (n = 86) | p-Value |
|----------------------|------------------------------|------------------------------|---------|
| **Age**              | 60.8 ± 6.3 (n = 9)           | 61.6 ± 6.1 (n = 68)          | 0.653   |
| **Ethnicity**        |                              |                              | 0.850   |
| Asian                | 0.0% (0/7)                   | 4.4% (2/45)                  |         |
| African American     | 14.3% (1/7)                  | 13.3% (6/45)                 |         |
| White                | 85.7% (6/7)                  | 82.2% (37/45)                |         |
| **PSA level**        | 17.5 ± 25.0 (n = 14)         | 14.0 ± 17.0 (n = 82)         | 0.626   |
| **Laterality**       |                              |                              | 0.703   |
| bilateral            | 92.9% (13/14)                | 89.4% (76/85)                |         |
| left                 | 0.0% (0/14)                  | 4.7% (4/85)                  |         |
| right                | 7.1% (1/14)                  | 5.9% (5/85)                  |         |
| **Gleason score**    |                              |                              | 0.398   |
| 6                    | 7.1% (1/14)                  | 9.3% (8/86)                  |         |
| 7                    | 64.3% (9/14)                 | 54.7% (47/86)                |         |
| 8                    | 0.0% (0/14)                  | 11.6% (10/86)                |         |
| 9                    | 21.4% (3/14)                 | 23.3% (20/86)                |         |
| 10                   | 7.1% (1/14)                  | 1.2% (1/86)                  |         |
| **M stage**          |                              |                              | 0.317   |
| M0                   | 92.9% (13/14)                | 100.0% (81/81)               |         |
| M1b                  | 7.1% (1/14)                  | 0.0% (0/81)                  |         |
| **T stage**          |                              |                              | 0.467   |
| T1c                  | 40.0% (4/10)                 | 51.4% (36/70)                |         |
| T2                   | 10.0% (1/10)                 | 1.4% (1/70)                  |         |
| T2a                  | 10.0% (1/10)                 | 5.7% (4/70)                  |         |
| T2b                  | 10.0% (1/10)                 | 8.6% (6/70)                  |         |
| T2c                  | 0.0% (0/10)                  | 15.7% (11/70)                |         |
| T3a                  | 20.0% (2/10)                 | 11.4% (8/70)                 |         |
| T3b                  | 10.0% (1/10)                 | 2.9% (2/70)                  |         |
| T4                   | 0.0% (0/10)                  | 2.9% (2/70)                  |         |
3.2. Genes Associated in ETV4-Fusion-Positive Prostate Cancer

The mRNA expression of ETV4 was higher in ETV4-fusion-positive prostate cancer, as compared to that in ETV4-fusion-negative prostate cancer. A Pearson correlation test using an absolute correlation coefficient value over 0.3 (|R| > 0.3) was applied to identify genes significantly correlated with ETV4 expression. A total of 393 genes were obtained, and interestingly, except for 10 genes, most were positively correlated (Table S1). These 393 genes were further analyzed using cancer-related genes from the CancerMine database, and 122 genes were defined as cancer-related genes (Table S1). Of these, 19 genes had previously reported oncogenic functions in prostate cancer (Table S1), which suggests that these genes could specifically function in ETV4-fusion-positive prostate cancers. Furthermore, the oncogenic role of approximately 100 of the cancer-related genes in ETV4-fusion-positive prostate cancers were suggested, and consistently, as an example, a recent study reported that CDK19 is highly expressed in prostate cancer and increases aggressiveness and regulates the progression of prostate cancer [14]. CDK19 was the cancer-related gene most highly correlated with ETV4 expression (Table S1), confirming its oncogenic function in ETV4-fusion-positive prostate cancer.

3.3. Cellular Signaling Pathways Associated with ETV4-Fusion-Positive Prostate Cancer

Cellular signaling pathway analysis was performed using the selected 393 genes via ORA to investigate altered signaling pathways in the ETV4-fusion-positive prostate cancer group. Six cancer-specific signaling pathways—The irinotecan pathway, metabolism, androgen receptor signaling, interferon signaling, MAPK/NF-kB signaling, and the tamoxifen pathway—were identified, and 86 genes were involved in these cancer-specific pathways (Figure 1). We further dissected the metabolic pathways and identified lipid metabolism, carbohydrate metabolism, and biosynthesis of cofactors, which were targeted by at least five genes (Figure S2).

A total of 85 genes were significantly highly expressed, and only PXN was downregulated in ETV4-fusion-positive prostate cancer (Figure S1a). Paxillin, encoded by the PXN gene, is an oncogenic protein. Paxillin relates to cancer proliferation and metastasis, and PXN gene expression is relatively high in many cancers, including prostate cancer [15–17]. PXN expression was relatively high in ERG1- and FLI1-fusion-positive prostate cancers and SPOP-mutant prostate cancer, but it was relatively low in ETV1- and ETV4-fusion-positive prostate cancers (Figure S1b). Consistently, a positive correlation between PXN and ERG (R = 0.5) and a negative correlation between ETV1/ETV4 and PXN (R = −0.3) were observed. Moreover, PXN expression in ETV4-fusion-positive prostate cancer was lower compared to normal (Figure S1b).

Gene-pathway network analysis was further performed (Figure 2). Six genes, CYP1B1, CYP3A4, HSPA5, RAN, SULT2A1, and TOP1, were involved in more than two pathways, and multiple targeted pathways included the tamoxifen pathway, metabolism, the irinotecan pathway, and androgen receptor signaling. Cellular signaling pathways altered in ETV4-fusion-positive cancer were validated by analyzing microarray data from a previous study [18]. In the study, 774 genes were altered by either overexpressing or knocking-down ETV4 in a prostate cancer cell line, and signaling pathways were analyzed using these genes. When ORA was performed using 774 genes, metabolism, interferon signaling, and MAPK/NF-kB signaling, among seven inferred pathways, were consistent with the results of this study using the TCGA database (Figure 3).
signaling, and MAPK/NF-κB signaling, among seven inferred pathways, were consistent with the results of this study using the TCGA database (Figure 3).

Figure 1. RNA expression heatmap of six cancer-specific pathways. RNA expression was converted into z-scores. Rows include each gene of the cancer-specific pathways arrayed by each pathway. Columns include each sample of an ETV4-fusion-positive or -negative group.
Figure 1. RNA expression heatmap of six cancer-specific pathways. RNA expression was converted into z-scores. Rows include each gene of the cancer-specific pathways arrayed by each pathway. Columns include each sample of an ETV4-fusion-positive or -negative group.

Figure 2. Gene-pathway network. Round rectangles are pathways, and ellipses are genes. The colors of the lines originate from the colors of each rectangle of the pathway.

Figure 3. Comparison of pathways found from two studies on ETV4-related changes. Pathway analysis was performed by CPDB. This study used 393 genes correlated with ETV4 expression, and the previous study used 774 genes altered by overexpression or knock-down of ETV4 [18].
3.4. Identifying Therapeutic Targets and Potential Actionable Drugs

To suggest therapeutic molecular targets and candidate drugs for ETV4-fusion-positive prostate cancer, gene–drug network analysis was performed using 86 genes involved in cancer-specific pathways of ETV4-fusion-positive prostate cancer. PARP1, NQO1, HSPA5, and TOP1 were identified as potential molecular targets for ETV4-fusion-positive prostate cancer, and olaparib, amrubicin, fluorouracil, and irinotecan were suggested as candidate drugs, respectively (Table 2). HSPA5 and TOP1 were associated with the irinotecan pathway and androgen receptor signaling, while PARP1 was categorized under the androgen receptor signaling pathway, and NQO1 was related to metabolism (Figure 1).

Table 2. Actionable drug list and targeted gene.

| Gene     | Signaling Pathway                        | Drug                                      |
|----------|------------------------------------------|-------------------------------------------|
| TOP1     | Irinotecan pathway                        | Irinotecan                                |
|          | Androgen receptor signaling               | Topotecan, Carboplatin, Cyclophosphamide  |
| HSPA5    | Irinotecan pathway                        | Fluorouracil                              |
|          | Androgen receptor signaling               |                                           |
| NQO1     | Metabolism                                | Amrubicin                                 |
| PARP1    | Androgen receptor signaling               | Olaparib                                  |

4. Discussion

ETV4 rearrangement has lately been discovered as a driver gene in 2% of prostate cancers. The small number of ETV4 subtypes among prostate patients and the short history of research on ETV4 rearrangement have limited understanding of the molecular features of ETV4 subtypes of prostate cancer and, in turn, have resulted in an absence of effective targeted therapy for these patients. In addition, since ETV4 is a transcription factor, it is difficult for it to be a direct drug target, unlike proteins that have a kinase domain or cell-surface receptor [4]. Considering that ETV4 rearrangement is mutually exclusive with other oncogenic driver mutations in prostate cancer, the study of the molecular features, specific therapeutic targets, and potential drugs for ETV4 subtypes of prostate cancer are needed. In this study, we first identified 393 genes correlated with ETV4 expression and six cancer-specific cellular signaling pathways, namely the irinotecan pathway, metabolism, androgen receptor signaling, interferon signaling, MAPK/NF-kB signaling and the tamoxifen pathway, altered by the presence or absence of ETV4 fusion. These signaling pathways could be further categorized into three groups: hormone-related pathways, metabolic pathways, and inflammation/cancer pathways.

Androgen signaling is stimulated by androgen, and activated androgen signaling multiplies and spreads prostate cancer cells [19]. Anti-androgen therapy is used to stop the growth of and shrink cancer cells [20,21]. We previously reported that ERG subtypes of prostate cancer have altered androgen receptor signaling, similar to the ETV4 subtypes of prostate cancer shown in the current study [22]. A previous study showed that anti-androgen therapy has a better effect on tumorigenesis in the ERG subtypes of prostate cancer [23], and we expect a similar effectiveness from anti-androgen therapy in ETV4-fusion-positive prostate cancer, based on its unique features of cellular signaling pathways. Estrogen treatment was once suggested as an alternative method to suppress testosterone in prostate cancer [24], and consistently, we found that the tamoxifen pathway was associated with ETV4-fusion-positive prostate cancer, and all genes related to the tamoxifen pathway were positively correlated with ETV4. These observations suggest the advantage of using hormonal therapy in ETV4-fusion-positive prostate cancer. As half of the genes included in the six cancer-specific pathways were related to the metabolism pathway, interestingly, the metabolic pathway was highly associated with the ETV4 subtype of prostate cancer (Figure 1). Metabolism has a close connection to cancer. When a normal prostate cell
transforms into a prostate cancer cell, many alterations in metabolism occur, including lipid and glucose metabolism [25–27]. Also, genes or mechanisms related to the metabolism pathway have been used as therapeutic targets [25,28]. This suggests that our selected genes in the metabolism pathway could be possible therapeutic targets for ETV4-fusion-positive cancer. MAPK/NF-κB signaling was also altered in the ETV4 subtype of prostate cancer (Figure 1). The MAPK and NF-κB pathways are common inflammatory signaling pathways. Activation of the MAPK and NF-κB pathways causes the release of proinflammatory cytokines, such as interleukin-6 (IL-6), IL-8, and tumor necrosis factor-α (TNF-α), which leads to the inflammatory response, and eventually causes cancer. Several studies have shown that inhibition of MAPK or NF-κB pathway activation decreases prostate tumor progression, such as proliferation, invasion, and migration, and increases apoptosis in various cancer types, including prostate cancer [29].

Next, we performed gene–drug network analysis and identified new therapeutic targets and drugs for ETV4-fusion-positive prostate cancer. Genes associated with ETV4-fusion-positive prostate cancers were used as the input, and four therapeutic candidate targets, PARP1, NQO1, HSPA5, and TOP1, and the respective selective drugs, olaparib, amrubicin, fluorouracil, and irinotecan, were identified. Olaparib, the first targeted drug for prostate cancer, has recently been approved for BRCA-mutated metastatic castration-resistant prostate cancer [30]. In addition, our study suggests that olaparib could also be actionable in the ETV4 subtype of prostate cancer, regardless of BRCA mutation. A large clinical study should be warranted for evaluation of the effects of olaparib on the ETV4 subtype of prostate cancer in the future.

Furthermore, we found different gene expression patterns in different types of prostate cancer. For example, PXN, which is known as an oncogene in prostate cancer [15], has higher expression in the ERG and SPOP subtypes of prostate cancer, as compared to normal cells, and lower expression in ET1V1 and ETV4 subtypes of prostate cancer, as compared to normal cells (Figure S1b). This shows that understanding the molecular features of each cancer subtype is a prerequisite for precision medicine. For example, PXN is known to be related with tumor progression, invasion, and metastasis via EMT [31–34], and the expression analysis in this study suggests that a prostate cancer subtype with ERG fusion or SPOP mutation could share this cancer progression mechanism while a prostate cancer subtype with an ETV4 fusion or ET1V1 fusion does not.

In this study, cellular signaling pathway analysis was extensively performed, and novel therapeutic targets and candidate drugs for ETV4-fusion-positive prostate cancer patients were systematically identified. Cancer genomic data, cancer transcriptomic data, clinical data, and patient information were obtained from the TCGA database. Microarray data performed in cell lines after upregulating or downregulating ETV4 was collected from prior study data and utilized to conclude the molecular function of ETV4. The future validation of the potential therapeutic targets and potential drugs in in vitro or in vivo models would demonstrate the efficiency of our approach.

ETV4 overexpression was observed in various cancer types, such as prostate cancer, bladder cancer, gastric cancer, colon cancer, and hepatocellular carcinoma, and the underlying mechanism and oncogenic function have been revealed, which suggest the importance of ETV4 in cancer research [35]. Therefore, our study will help to advance and accelerate the understanding ETV4-associated human cancers.

5. Conclusions

In this study, we discovered that six cancer-specific cellular signaling pathways, the irinotecan pathway, metabolism, androgen receptor signaling, interferon signaling, MAPK/NF-κB signaling, and the tamoxifen pathway, were altered in the ETV4 subtype of prostate cancer. To suggest potential therapeutic targets and actionable candidate drugs, gene–drug network analysis was performed, and four genes, HSPA5, NQO1, PARP1, and TOP1, and their targeted drugs, fluorouracil, amrubicin, olaparib, and irinotecan, respectively, were identified.
Taken together, we have provided information on altered cellular signaling pathways and therapeutic targets for ETV4-fusion-positive prostate cancer. Considering the absence of targeted drugs for the ETV4 subtype of prostate cancer, our study will allow a step forward in investigations of treatments. Furthermore, the analysis algorithms we established in this study could be applied to analyze the molecular features of other types of cancer, and in turn, this could provide enormous benefits in developing new drug treatments in regard to cost, time, and the prediction of adverse effects.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines10102650/s1: Table S1: Information about genes correlated with ETV4; Figure S1: Pattern of PXN expression in prostate cancer; Figure S2: Gene-pathway network about the metabolism pathway.

Author Contributions: Conceptualization, Y.J.S. and H.S.K.; methodology, Y.J.S. and J.W.Y.; formal analysis, Y.J.S.; investigation, Y.J.S., J.W.Y. and H.S.K.; data curation, Y.J.S. and J.W.Y.; writing—original draft preparation, Y.J.S.; writing—review and editing, H.S.K. and J.W.Y.; visualization, Y.J.S.; supervision, H.S.K. and J.W.Y.; funding acquisition, H.S.K. and J.W.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (NRF-2021R1A2C2008271, NRF-2022R1C1C1012986 and NRF-2021M3E5D7079872), and by a VHS Medical Center Research Grant from Korea (VHSMC22057).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA A Cancer J. Clin. 2021, 71, 209–249. [CrossRef] [PubMed]
2. Kumar-Sinha, C.; Tomlins, S.A.; Chinnaiyan, A.M. Recurrent Gene Fusions in Prostate Cancer. Nat. Rev. Cancer 2008, 8, 497–511. [CrossRef] [PubMed]
3. Tomlins, S.A.; Rhodes, D.R.; Perner, S.; Dhanasekaran, S.M.; Mehra, R.; Sun, X.-W.; Varambally, S.; Cao, X.; Tchinda, J.; Kuefer, R.; et al. Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer. Science 2005, 310, 644–648. [CrossRef] [PubMed]
4. Tomlins, S.A.; Bjartell, A.; Chinnaiyan, A.M.; Jenster, G.; Nam, R.K.; Rubin, M.A.; Schalken, J.A. ETS Gene Fusions in Prostate Cancer: From Discovery to Daily Clinical Practice. Eur. Urol. 2009, 56, 275–286. [CrossRef]
5. Scaravilli, M.; Koivukoski, S.; Latonen, L. Androgen-Driven Fusion Genes and Chimeric Transcripts in Prostate Cancer. Front. Cell Dev. Biol. 2021, 9, 623809. [CrossRef]
6. Tomlins, S.A.; Mehra, R.; Rhodes, D.R.; Smith, L.R.; Roulston, D.; Helgeson, B.E.; Cao, X.; Wei, J.T.; Rubin, M.A.; Shah, R.B.; et al. TMPRSS2:ETV4 Gene Fusions Define a Third Molecular Subtype of Prostate Cancer. Cancer Res. 2006, 66, 3396–3400. [CrossRef]
7. Yun, J.W.; Yang, L.; Park, H.-Y.; Lee, C.-W.; Cha, H.; Shin, H.-T.; Noh, K.-W.; Choi, Y.-L.; Park, W.-Y.; Park, P.J. Dysregulation of Cancer Genes by Recurrent Intergenic Fusions. Genome Biol. 2020, 21, 166. [CrossRef]
8. Pellecchia, A.; Pescucci, C.; De Lorenzo, E.; Luceri, C.; Passaro, N.; Sica, M.; Notaro, R.; De Angioletti, M. Overexpression of ETV4 Is Oncogenic in Prostate Cells through Promotion of Both Cell Proliferation and Epithelial to Mesenchymal Transition. Oncogenesis 2012, 1, e20. [CrossRef]
9. Aytes, A.; Mitrofanova, A.; Kinkade, C.W.; Lefebvre, C.; Lei, M.; Phelan, V.; LeKaye, H.C.; Koutcher, J.A.; Cardiff, R.D.; Califano, A.; et al. ETV4 Promotes Metastasis in Response to Activation of PI3-Kinase and Ras Signaling in a Mouse Model of Advanced Prostate Cancer. Proc. Natl. Acad. Sci. USA 2013, 110, E3506–E3515. [CrossRef]
10. Abeshouse, A.; Ahn, J.; Akbani, R.; Ally, A.; Amin, S.; Andry, C.D.; Annala, M.; Aprikian, A.; Armenia, J.; Arora, A.; et al. The Molecular Taxonomy of Primary Prostate Cancer. Cell 2015, 163, 1011–1025. [CrossRef]
11. Lever, J.; Zhao, E.Y.; Grewal, J.; Jones, M.R.; Jones, S.J.M. CancerMine: A Literature-Mined Resource for Drivers, Oncogenes and Tumor Suppressors in Cancer. Nat. Methods 2019, 16, 505–507. [CrossRef] [PubMed]
12. Gu, Z.; Eils, R.; Schlesner, M. Complex Heatmaps Reveal Patterns and Correlations in Multidimensional Genomic Data. Bioinformatics 2016, 32, 2847–2849. [CrossRef] [PubMed]
13. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res. 2003, 13, 2498-2504. [CrossRef] [PubMed]

14. Pan-Cancer Analysis of the Mediator Complex Transcriptionome Identifies CDK19 and CDK8 as Therapeutic Targets in Advanced Prostate Cancer | Clinical Cancer Research | American Association for Cancer Research. Available online: https:// AACR Journals. org/cancerres/article/23/7/1829/80365/Pan-Cancer-Analysis-of-the-Mediator-Complex (accessed on 9 July 2022).

15. Sen, A.; Castro, I.D.; DeFranco, D.B.; Deng, F.-M.; Melamed, J.; Kapur, P.; Raj, G.V.; Rossi, R.; Hammes, S.R. Paxillin Mediates Extracellular and Intracellular Signaling in Prostate Cancer Proliferation. J. Clin. Investig. 2012, 122, 2469-2481. [CrossRef]

16. Chen, D.; Wang, Z.; Ren, C.; Zeng, Z.; Wang, D.; Luo, H.; Wang, F.; Qiu, M.; Bai, L.; Zhang, D.; et al. Abnormal Expression of Paxillin Correlates with Tumor Progression and Poor Survival in Patients with Gastric Cancer. J. Transl. Med. 2013, 11, 277. [CrossRef]

17. Liu, Q.; Wang, J.; Tang, M.; Chen, L.; Qi, X.; Li, J.; Yu, J.; Qiu, H.; Wang, Y. The Overexpression of PXN Promotes Tumor Progression and Leads to Radiosensitivity in Cervical Cancer. Future Oncol. 2018, 14, 241-253. [CrossRef]

18. Hollenhorst, P.C.; Paul, L.; Ferris, M.W.; Graves, B.J. The ETS Gene ETV4 Is Required for Anchorage-Independent Growth and a Cell Proliferation Gene Expression Program in PC3 Prostate Cells. Genes Cancer 2010, 1, 1044–1052. [CrossRef]

19. Lonergan, P.E.; Tindall, D.J. Androgen Receptor Signaling in Prostate Cancer Development and Progression. J. Carcinog. 2011, 10, 20. [CrossRef]

20. Chen, Y.; Clegg, N.J.; Scher, H.I. Anti-Androgens and Androgen-Depleting Therapies in Prostate Cancer: New Agents for an Established Target. Lancet Oncol. 2009, 10, 981-991. [CrossRef]

21. Clegg, N.J.; Wongvipat, J.; Joseph, J.D.; Tran, C.; Ouk, S.; Dilhas, A.; Chen, Y.; Grillot, K.; Bischoff, E.D.; Cai, L.; et al. ARN-509: A Novel Antiandrogen for Prostate Cancer Treatment. Cancer Res. 2012, 72, 1494-1503. [CrossRef]

22. Yun, J.W.; Lee, S.; Chun, S.; Lee, K.W.; Kim, J.; Kim, H.S. Comprehensive Analysis of Oncogenic Signatures and Consequent Repurposed Drugs in TMPRSS2-ERG Fusion-positive Prostate Cancer. Clin. Transl. Med. 2021, 11, e420. [CrossRef] [PubMed]

23. Graff, R.E.; Pettersson, A.; Lis, R.T.; Preu, N.; Jordahl, K.M.; Nulttall, E.; Rider, J.R.; Fiorentino, M.; Sesso, H.D.; Kenfield, S.A.; et al. The TMPRSS2-ERG Fusion and Response to Androgen Deprivation Therapy for Prostate Cancer. Prostate 2015, 75, 897-906. [CrossRef] [PubMed]

24. Crawford, E.D. Hormonal Therapy in Prostate Cancer: Historical Approaches. Rev. Urol. 2004, 6, S3–S11. [PubMed]

25. Flavin, R.; Zadra, G.; Loda, M. Metabolic Alterations and Targeted Therapies in Prostate Cancer. J. Pathol. 2011, 223, 284–295. [CrossRef]

26. Cutruzzola, F.; Giardina, G.; Marani, M.; Macone, A.; Paiardini, A.; Rinaldo, S.; Paone, A. Glucose Metabolism in the Progression of Prostate Cancer. Front. Physiol. 2017, 8, 97. [CrossRef]

27. Wu, X.; Daniels, G.; Lee, P.; Monaco, M.E. Lipid Metabolism in Prostate Cancer. Am. J. Clin. Exp. Urol. 2014, 2, 111–120.

28. Li, X.; Wu, K.; Fan, D. CIAPIN1 as a Therapeutic Target in Cancer. Expert Opin. Ther. Targets 2010, 14, 603–610. [CrossRef]

29. Wang, C.Y.; Mayo, M.W.; Baldwin, A.S. TNF- and Cancer Therapy-Induced Apoptosis: Potentiation by Inhibition of NF-KappaB. Mol. Ther. Oncolytics 2020, 10, 382-387. [CrossRef]

30. De Bono, J.; Mateo, J.; Fizazi, K.; Saad, F.; Shore, N.; Oudard, S.; Fizazi, K.; et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N. Engl. J. Med. 2020, 382, 2091–2102. [CrossRef]

31. Wen, L.; Zhang, X.; Zhang, J.; Chen, S.; Ma, Y.; Hu, J.; Yue, T.; Wang, J.; Zhu, J.; Wu, T.; et al. Paxillin Knockdown Suppresses Metastasis and Epithelial-mesenchymal Transition in Colorectal Cancer via the ERK Signalling Pathway. Oncol. Rep. 2020, 44, 1105–1115. [CrossRef]

32. Gu, H.; Wen, J. Abnormal Level of Paxillin in Cervical Cancer Cells Is Involved in Tumor Progression and Invasion. Acta Biochim. Pol. 2021, 68, 49–53. [CrossRef] [PubMed]

33. Liu, X.; Xu, D.; Xu, X.; Xue, Q.; Gao, X.; Tang, C. MiR-216b Regulates the Tumorigenesis of Gastric Cancer by Targeting PXN. Pathol. Res. Pract. 2021, 218, 153325. [CrossRef] [PubMed]

34. Tanaka, N.; Minemura, C.; Asai, S.; Kikikawa, N.; Kinoshita, T.; Oshima, S.; Koma, A.; Kasamatsu, A.; Hanazawa, T.; Uzawa, K.; et al. Identification of MiR-199-5p and MiR-199-3p Target Genes: Paxillin Facilities Cancer Cell Aggressiveness in Head and Neck Squamous Cell Carcinoma. Genes 2021, 12, 910. [CrossRef] [PubMed]

35. Jiang, W.; Xu, Y.; Chen, X.; Pan, S.; Zhu, X. E26 Transformation-Specific Variant 4 as a Tumor Promotor in Human Cancers through Specific Molecular Mechanisms. Mol. Ther. Oncolytics 2021, 22, 518–527. [CrossRef]