Systematic profiling of alternative splicing signature reveals prognostic predictor for prostate cancer

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Abstract

Alternative splicing (AS) provides the primary mechanism for producing protein diversity. There is growing evidence that AS is involved in the development and progression of cancers. The rapid accumulation of high-throughput sequencing technologies and clinical data sets offers an opportunity to systematically profile the relationship between mRNA variants and clinical outcomes. However, there is a lack of systematic analysis of AS in prostate cancer: Download RNA-seq data and clinical information from The Cancer Genome Atlas (TCGA) data portal. Evaluate RNA splicing patterns by SpliceSeq and calculate splicing percentage (PSI) values. Different expressions were identified as differently expressed AS events (DEAs) based on PSI values. Bioinformatics methods were used for further analysis of DEAs and their splicing networks. Kaplan-Meier, Cox proportional regression, and unsupervised cluster analysis were used to assess the correlation between DEAs and clinical characteristics. In total, 43,834 AS events were identified, of which 1,628 AS events were differentially expressed. The parental genes of these DEAs played a significant role in the regulation of prostate cancer-related processes. In total, 226 DEAs events were found to be associated with disease-free survival. Four clusters of molecules with different survival modes were revealed by unsupervised cluster analysis of DEAs. AS events may be important determinants of prognosis and bio-modulation in prostate cancer. In this study, we established strong prognostic predictors, discovered a splicing network that may be a potential mechanism, and provided further validated therapeutic targets.

KEYWORDS
alternative splicing, prognosis, prostate cancer, RNA-seq

1 | INTRODUCTION
Alternative splicing of RNA refers to the process by which mRNA precursors are cleaved at different sites to produce different mRNA splicing bodies, which are then translated into different proteins with different functions. Alternative splicing is an important factor in the transcriptomics and proteomics diversification of eukaryotes. Studies have shown that 95% of human

Abbreviations: AA, alternate acceptor site; AD, alternate donor site; AP, alternate promoter; AR, androgen receptor; AR-V7, androgen receptor splice variant 7; AS, alternative splicing; AT, alternate terminator; AUC, area under the curve; CRPC, castration-resistant prostate cancer; DEAs, differentially expressed alternative splicing events; DFS, disease-free survival; ES, exon skip; GO, gene ontology; HR, hazard ratio; M, metastasis; ME, mutually exclusive exons; N, lymph node; PSI, percent spliced in; RI, retained intron; ROC, receiver operating characteristic; T, tumor; TCGA, The Cancer Genome Atlas; VEGF, vascular endothelial growth factor.
genes could be spliced. In recent years, increasing evidence has suggested that splicing defects and the production of specific isomers are drivers for cancer. Furthermore, AS is closely correlated with tumor proliferation, apoptosis, hypoxia, angiogenesis, immune escape, and metastasis. Conversely, mutations or changes in the expression of the splicing factor may result in specific cancer-promoting splicing bodies, providing growth or survival advantages for the tumor cells. Therefore, abnormal AS is considered to be another marker of cancer, and systematic studies of AS may provide potential biomarkers for malignant tumors.

Prostate cancer is one of the most common malignant tumors in the male reproductive system, occurring mainly in the elderly (age > 65 y) and ranking second among male cancer-related deaths. The importance of AS in the progression of prostate cancer has been demonstrated in many studies. Growing evidence suggests that splicing events and dysregulation of cancer-specific splicing variants can serve as prognostic biomarkers and therapeutic targets for prostate cancer. Therefore, identifying the link between splicing disorders and prostate cancer, especially accurate molecular analysis, is a key issue in ongoing cancer research. Therefore, there is an urgent need to exploit the genome-wide transcriptome approach to develop the potential prognostic value of AS events in patients with prostate cancer.

With the rapid accumulation of RNA-seq data in prostate cancer samples, TCGA project provides a rich source for AS model research. At the same time, TCGA database provides detailed clinical information. Therefore, it is possible to correlate prostate cancer-associated AS events in a larger population. RNA-seq has great advantages for the study of cancer, but requires efficient and reliable bioinformatics for data mining. The recently developed analysis tool, SpliceSeq, aligns RNA-seq readings with gene maps for accurate analysis of complex or low frequency AS events.

We used RNA-seq data from TCGA program data to elucidate the role of differential alternative splicing patterns in 433 prostate cancer cohorts and further systematically examined the potential prognostic impact of prostate cancer-specific AS events on patient survival. The aim of this study was to explore differential RNA splicing patterns in prostate cancer and to elucidate the function of splicing variants as prognostic biomarkers. This research will help to develop new therapeutic targets for prostate cancer.

2 | MATERIALS AND METHODS
2.1 | Alternative splicing event in TCGA RNA-seq data

TCGA Data Portal (https://portal.gdc.cancer.gov/projects) provided RNA-seq data for TCGA prostate cancer. We used the SpliceSeq tool to analyze AS profiles and to assess the splicing pattern of genes encoding protein in patients with prostate cancer. The PSI values, from 0 to 1, were used to quantify AS events and calculate 7 types of variable splicing events: ES, ME, RI, AP, AT, AD, and AA. The expression data of prostate cancer, relevant clinical information (DFS > 30 d), and variable splicing data were integrated. Finally, 433 prostate cancer samples and 46 normal control samples were selected for subsequent study and analysis.

2.2 | Differential analysis of alternative splicing events

In this study, a comparison was made between the prostate cancer samples and the adjacent normal AS samples to identify differentially alternative splicing events, and $P < .05$ and $|\text{Log FC}|>1$ were defined as differential alternative splicing events. At the same time, we also analyzed the gene expression profiles of prostate cancer samples and normal cancer samples, and identified differentially expressed genes for further comparison.

2.3 | Survival analysis

In total, 433 patients with prostate cancer with DFS exceeding 30 d were included in the study of survival analysis. Patients were divided into 2 groups based on the median cutoff value for each parameter. Univariate Cox regression was performed on 7 types of candidate variable splicing events to identify the relationship between AS events and DFS. A $P$-value less than .05 was defined as an AS event associated with survival. At the same time, we used the clusterProfiler package to perform GO function enrichment analysis on survival-related AS events, and identified significantly related biological processes, cellular components, and molecular functions. To remove any gene that may not be an independent factor in prognostic predictors, multivariate Cox regression was further applied to the survival-related variable splicing events in 7 types. Finally, 7 different types of candidate independent prognostic AS events were combined to construct a final prognostic predictor. In addition, we plotted the ROC curve to compare the accuracy of prognostic models in each type of AS.

2.4 | Splicing related network construction

A list of 67 human splicing factors was created through hand-planned literature and database screening. The expression of the splicing factor gene in the mRNA splicing pathway was derived from level 3 mRNA-seq data in TCGA. Survival analysis was used to identify survival-related splicing factors, and Spearman test was used to analyze the correlation between survival-related splicing factor gene expression and survival-related AS PSI values. When the $P$-value was less than .05, it was defined as a significant correlation and Cytoscape (v.3.6.0) software was used to construct the final interaction network.
of AS events and splicing factors. At the same time, we used clusterProfiler to perform GO functional enrichment analysis on genes associated with AS networks to identify significantly correlated biological processes, cellular components, and molecular functions.

2.5 Identify prognosis and analyze subtype-related clusters

Alternative splicing events occurred very differently at the individual level. To obtain robust classification, we used the unsupervised consensus approach implemented by Consensus Cluster Plus (R package) to identify molecular subtypes of prostate cancer. We analyzed survival of the identified molecular subtypes from the survival time and then identified the relationship between subtypes and survival, and further explored the relevant clinical information, trying to find a relationship between other clinical information and molecular subtypes. Details of this study design are illustrated as a flowchart in Figure 1.

3 RESULTS

3.1 Integrating alternative splicing events in prostate cancer

Of the 433 prostate cancer patients, 43,834 AS events associated with 10,308 genes were found. We detected 3,503 AA-type AS events containing 2,469 genes, 3,088 AD-type AS events involving 2,175 genes, 9,003 AP-type AS events including 3,608 genes, 8,620 AT-type AS events involving 3,761 genes, 16,665 ES-type AS events containing 6,530 genes, 227 ME-type AS events involving 220 genes, 2,728 RI type of AS event containing 1,833 genes, as shown in Figure 2A. The results indicated that one gene may have several types of mRNA splicing events, and one gene may be expressed by 4 AS types, while ES was the main type, because more than one-third of the AS events were ES events.

3.2 Differential alternative splicing events in prostate cancer

Differential AS analysis revealed that 1,628 differential AS events were identified in prostate cancer involving 1,326 genes, of which 1,338 were differentially upregulated AS events. Also, 290 were differentially downregulated AS events, with upregulated events in the main body, as shown in Figure 2B, in which the upregulated and downregulated TOP 15 differential mutagenic events-related expression and partial event cancer were compared with normal samples, as shown in Figure 2C,D and Table 1. Some genes could be expressed in up to 4 AS types, as shown in Figure 2E. Differential expression profiling revealed that 2,928 differential expression genes were identified in prostate cancer. We compared the genes involved in the differential AS event with the differentially expressed genes, and found that the genes recognized in both had similarities, but there were also significant differences; the AS events could make up for shortcomings of differential expression analysis, as shown in Figure 2F.
**Figure 2**

A. Overview of alternative splicing (AS) events and their interactions between genes. One gene may have several types of AS.

B. Difference of AS events. Volcano plot visualizing the DEAs identified in prostate cancer. The red and blue points in the plot represent the differentially expressed AS with statistical significance.

C. Heat map of the AS events. The horizontal axis shows the clustering information of samples which were divided into 2 major clusters, and the clusters were normal tissue and paired tumor tissue, respectively (Top 15 events).

D. Boxplot of the difference of AS events between normal tissue and paired tumor tissue (Top 3 events).

E. UpSet plot of different AS events and their interactions between genes.

F. Venn diagram demonstrated the intersection set of gene and different alternative splicing events.
To investigate the prognostic value of AS events in patients with prostate cancer, we used univariate Cox regression analysis to assess the prognostic impact of differential AS events in patients with prostate cancer. We detected a total of 226 survival-related AS events ($P < 0.05$) among the differential alternative splicing events, as shown in Figure 3A. It can be further seen, among the recognized survival-related AS events, that adverse factors (HR > 1) were found more frequently than protective factors (HR < 1), either for the whole or for each type, as shown in Figure 3B. The Circos diagram shows details of survival-related AS events and their associated genes, as seen in Figure 3C. We drew forest maps by spotting the TOP 12 most important survival-related AS events among 6 AS types (AS events associated with survival were not found in the ME type), as shown in Figure 3D-I. Subsequently, GO function enrichment analysis was performed on 211 genes involved in survival-related AS events, and biological processes, cell components, and molecular functions ($P < 0.05$) with significant concentrations of AS events were found; biological processes with significant representation are shown in Figure 3J.

TABLE 1

| Symbol  | AS type | Exons | MeanT | MeanN | logFC  | P-value |
|---------|---------|-------|-------|-------|--------|---------|
| GSTM2   | AT      | 13    | 0.240 | 0.035 | 0.832  | 9.45E-59 |
| COL6A3  | AT      | 6     | 0.273 | 0.104 | 0.417  | 3.18E-56 |
| COMT    | AP      | 3     | 0.179 | 0.027 | 0.828  | 2.68E-54 |
| L3MBTL4 | AT      | 19    | 0.330 | 0.150 | 0.342  | 4.45E-48 |
| ALDH1L1 | AP      | 1     | 0.660 | 0.059 | 1.046  | 3.08E-44 |
| GABARAP | RI      | 1.2:1.3:1.4 | 0.021 | 0.006 | 0.549  | 3.94E-43 |
| ZNF331  | AP      | 1     | 0.285 | 0.099 | 0.460  | 5.96E-42 |
| COL4A6  | AT      | 52    | 0.135 | 0.021 | 0.801  | 2.03E-41 |
| PRUNE2  | AT      | 7     | 0.089 | 0.002 | 1.586  | 9.68E-41 |
| ANO6    | AT      | 25    | 0.087 | 0.032 | 0.432  | 9.55E-40 |
| LGALS3  | AT      | 6     | 0.076 | 0.034 | 0.346  | 1.54E-38 |
| EMP2    | AP      | 2     | 0.224 | 0.055 | 0.614  | 5.14E-38 |
| MATR3   | AP      | 1     | 0.084 | 0.025 | 0.527  | 3.69E-37 |
| CKMT2   | ES      | 2     | 0.283 | 0.096 | 0.472  | 7.88E-37 |
| MAGED2  | AP      | 3     | 0.167 | 0.036 | 0.670  | 2.21E-35 |

| Symbol  | AS type | Exons | MeanT | MeanN | logFC  | P-value |
|---------|---------|-------|-------|-------|--------|---------|
| ALDH1L1 | AP      | 3     | 0.281 | 0.766 | −0.435 | 1.19E-31 |
| MTUS2   | AP      | 7     | 0.140 | 0.711 | −0.706 | 2.69E-23 |
| OSBPS5  | AP      | 1.1   | 0.345 | 0.728 | −0.324 | 4.02E-20 |
| VPS9D1  | AP      | 1     | 0.266 | 0.597 | −0.351 | 4.05E-17 |
| RGS12   | AP      | 7.1   | 0.226 | 0.529 | −0.370 | 6.46E-17 |
| PAK6    | AP      | 2     | 0.112 | 0.450 | −0.602 | 1.16E-16 |
| DNMT3A  | AT      | 5.2   | 0.127 | 0.278 | −0.340 | 3.41E-15 |
| PRKAR1B | AP      | 5     | 0.127 | 0.341 | −0.427 | 2.22E-14 |
| NFYC    | AP      | 4     | 0.068 | 0.151 | −0.349 | 2.87E-14 |
| KRT222  | AT      | 5.2   | 0.016 | 0.073 | −0.652 | 3.91E-14 |
| TRIM16  | AP      | 6.1   | 0.251 | 0.529 | −0.324 | 4.05E-14 |
| RGS3    | AP      | 24    | 0.111 | 0.267 | −0.380 | 9.32E-14 |
| PDLIM5  | AT      | 4     | 0.031 | 0.069 | −0.344 | 1.29E-13 |
| CTAGE5  | AP      | 1     | 0.092 | 0.267 | −0.461 | 3.01E-13 |
| COX17   | AT      | 7     | 0.098 | 0.24  | −0.391 | 0.00   |

Note: The P-value was calculated by t test.
Abbreviations: logFC, log2 fold change; MeanN, the mean PSI value in paired normal tissue; MeanT, the mean PSI value in prostate cancer tissue.

3.3 Alternative splicing events associated with survival in prostate cancer

To investigate the prognostic value of AS events in patients with prostate cancer, we used univariate Cox regression analysis to assess the prognostic impact of differential AS events in patients with prostate cancer. We detected a total of 226 survival-related AS events ($P < 0.05$) among the differential alternative splicing events, as shown in Figure 3A. It can be further seen, among the recognized survival-related AS events, that adverse factors (HR > 1) were found more frequently than protective factors (HR < 1), either for the whole or for each type, as shown in Figure 3B. The Circos diagram shows details of survival-related AS events and their associated genes, as seen in Figure 3C. We drew forest maps by spotting the TOP 12 most important survival-related AS events among 6 AS types (AS events associated with survival were not found in the ME type), as shown in Figure 3D-I. Subsequently, GO function enrichment analysis was performed on 211 genes involved in survival-related AS events, and biological processes, cell components, and molecular functions ($P < 0.05$) with significant concentrations of AS events were found; biological processes with significant representation are shown in Figure 3J.
To detect independent prognostic factors in patients with prostate cancer, we selected survival-related AS events as candidate factors, and multivariate Cox regression analysis was used to identify independent prognostic factors among the retained 6 AS types (P < .05). One independent prognostic factor associated with AA, 4 independent prognostic factors associated with AD, 19 independent prognostic factors associated with AP, 11 independent prognostic factors associated with AT, 8 independent prognostic factors associated with ES, and 5 independent prognostic factors associated with RI were obtained. Subsequently, 6 different types of independent prognostic AS events were combined to construct a final prognostic predictor. In our data analysis of each type of splicing pattern, the ability to predict the results of patients with prostate cancer using prognostic models constructed by different types of AS events is shown in Figure 4A-F. In particular, a prognostic model constructed by a single AP model showed the maximum prediction power among the 6 prognostic models (ROC = 0.815). In addition, a final prognostic predictor was constructed by combining 6 different types of candidate independent prognostic AS events (a total of 48 alternative splicing events). Notably, the final prognosis model showed good performance to predict power (ROC = 0.868), as shown in Figure 4G-I.
FIGURE 5  A, B, Kaplan-Meier curves for splicing factors; C, Alternative splicing (AS) events network. The genes involved in downregulating splicing factors were represented with blue/green nodes. Red nodes were upregulating splicing factors. The positive/negative correlation between expressions of splicing factors and were represented with red/gray lines; D, E, Two cases for the relationship between PSI values of AS and the expression value of splicing factors; F, Gene network construction and functional enrichment analysis.
3.5  Interaction network between survival-related AS events and splicing factors

To determine which splicing factors were associated with survival-related AS events in prostate cancer, we performed a survival analysis of the splicing factor based on gene expression. The results showed that 16 splicing factors were significantly associated with overall survival, as seen in Figure 5A.B. Meanwhile, we also found that the vast majority (13/16) of survival-related splicing factors demonstrated that overexpression was associated with poor prognosis in patients. In addition, the correlation between the PSI values of prognosis-related AS events and the expression of survival-related splicing factors was investigated using Spearman test. Among them, 16 survival-related splicing factors (green dots) were significantly associated with 48 prognosis-related AS events (corresponding to 46 genes, 3 of which were downregulated and involved in alternative splicing events [blue dots] and 43 upregulated [red dots]) and 621 alternative splicing network interactions were constructed (the red line represents a positive correlation, the gray line represents a negative correlation, and the red line is dominant), as shown in Figure 5C. The correlation between the splicing factor hnRNP P and the AS event was reached 46 times, and genes such as ADAM15, ARAP1, IP6K2, SL2C5A29, SL6A8, TAF6, and WNT2B interacted with up to 16 splicing factors. The correlation between the splicing factor hnRNP P and ADAM15 and ARAP1 is shown in Figure 5D,E.

Subsequently, 62 genes involved in the AS network were profiled by GO functional enrichment analysis. Biological processes, cell components, and molecular functions (P < .05) were significantly represented among AS events, biological processes are shown in Figure 5F.

3.6  Molecular subtype cluster associated with prognosis

We further identified different AS patterns using unsupervised analysis of all samples based on AS events associated with prognosis. By combining the Elbow method to determine the optimal number of clusters, and based on the distribution of their consensus values ranging from 0 (white, samples never gathered together) to 1 (dark blue, samples always gathered together), we finally determined the 4 groups (C1, C2, C3, and C4) of samples as follows: C1 (n = 126, 29.1%), C2 (n = 102, 23.6%), C3 (n = 160, 36.9%), and C4 (n = 45, 10.4%), as shown in Figure 6A.B. Then we performed a Kaplan-Meier analysis to assess the relationship between clustering and prognosis. The results showed that clusters were associated with different survival patterns, with cluster 1 and cluster 2 correlating with poor outcomes in survival analysis, while cluster 3 and cluster 4 correlated with relatively good survival results, as shown in Figure 6C. At the same time, we further analyzed the relevant clinical information (such as survival time [DFS > 5 y or <5 y], survival status [alive or dead], T, N, M, age [age > 50 or < 50] and other information), finding that some related information, such as significant difference in survival status (alive or dead), T, and N, in the 4 clusters was not randomly distributed (chi-square test, P < .05), as shown in Figure 6D. Therefore, we could also identify molecular subtype clusters associated with prognosis through AS events.

4  DISCUSSION

Most of the genes in the human genome are composed of multiple exons and introns, and the introns are cleaved to produce mature mRNA, which in turn encodes a protein.18 The AS of RNA greatly enhances the diversity of the proteome, not only participating in normal physiological activities, but also playing a key role in the initiation or maintenance of human diseases, including prostate cancer.19-21 Its development is complicated, especially in advanced prostate cancer. During the 14-30 mo after receiving hormonal therapy, most patients will be converted to CRPC. Many AS events are involved in this process. For example, splicing variants of AR are identified in metastatic tumors of CRPC patients,22-24 the most widely studied is androgen receptor splice variant 7 (AR-V7), the expression of which is significantly associated with increased biochemical recurrence25 and decreased survival in patients.26-28 In addition, studies have shown the appearance of AS events in prostate cancer VEGF. The VEGF gene was thought to encode a single protein, but it is now very clear that the human VEGF gene generally contains 8 exons, producing at least 12 VEGF isomers by alternatively splicing within exons 6, 7 and 8,29 resulting in proteins with different heparin-binding properties.30 Furthermore, studies have shown that the ratio of different VEGF isoforms affects the formation of blood vessels in prostate cancer cells.31 In addition, studies on abnormal AS in prostate cancer-related genes (such as KLF6, BCL2L1, ERG, etc) are also frequently reported.32-33 Overall, the results confirmed that the AS expression of prostate cancer-associated genes and the balance of their splicing subtypes require wide and extensive research, because this approach could greatly expand the range of screening potential biomarkers and therapeutic targets.

In recent years, with the rapid development of high-throughput sequencing technologies and the continuous reduction in their cost, the diversity of AS maps for prostate cancer has achieved great success. Wang and colleagues34 pointed out that differences in AA splicing variants of PIK3CD, FGFR3, TSC2, and RASGRP2 showed stronger carcinogenicity. It was also pointed out that AS of the PIK3CD-S gene was closely related to poor prognosis of patients. Of course, the current TCGA database possesses a large amount of RNA-seq data from different cancer types. Through data mining, we can also systematically study RNA splicing disorders in cancer. By comparing different alternative splicing events between tumors and adjacent normal tissues, it has been established that survival-related AS events are associated with a variety of tumors, such as glioblastoma,35 breast cancer,36 lung cancer,37 and colorectal cancer.38 Through the analysis of TCGA database, we identified AS events and regulatory splicing factors in prostate cancer to fully understand their differential RNA splicing pattern. In total, 1628 differentially
AS events were identified, of which 1338 were upregulated and 290 were downregulated, involving 1326 genes.

We then systematically studied the relationship between AS events and survival period of patients with prostate cancer. In total, 226 AS events were significantly associated with survival rate of patients with prostate cancer. Splicing events such as for IP6K2, RPS6, DPM3, NDUFA13, TPM1, and WNT2B play a key role in tumor biology. For example, tropomyosin is an important regulatory protein in muscle contraction. In nonmuscle cells, TPM1 acts as a cell transformation inhibitor. Many of the splicing isoforms of TPM1, including the AT-splicing variant of TPM1 were found in our analysis to be involved in various types of tumors, such as head-and-neck cancer, esophageal cancer, breast cancer, and lung cancer. At the same time, for the first time, we found that high expression of the steroid-assisted regulatory gene CALCOCO1 AS RI was significantly associated with poor prostate cancer prognosis. This result is similar to findings for breast cancer, which is also hormone related.
In addition, we constructed a predictive model for each splicing type using multivariate Cox regression. The final predictive model for each splicing type performed quite well in distinguishing between patients with prostate cancer and their prognosis. The predictive models for each splicing type had different AUC values, and the prognostic model constructed by the AP model was the best among the 6 prognostic models, with the best predictive power (ROC = 0.815). We further combined 6 different types of candidate independent prognostic AS events to construct a final prognostic predictor with a predictive power (ROC = 0.868), which could provide patients with new predictive information and facilitate patient assessment.

Given that the regulation of key splicing factors may result in changes in widespread AS events, we further analyzed survival-related AS splicing factors and their associated networks and found that 16 splicing factors were significantly associated with overall patient survival. Interestingly, most of the splicing factor expression levels associated with poor survival were found for genes that were upregulated. Among them, the correlation between the splicing factor hnRNP P and the AS event was 46 times, which confirmed its key role in the development of prostate cancer, indicating that the results were consistent with the conclusions by Fei45 and so on, and providing the possibility to explore the development and treatment of prostate cancer.

To the best of our knowledge, our study is the first to describe different prognostic molecular subtypes These subtypes were divided into 4 groups of molecular clusters, the first 2 subtypes of which were related to poor survival of prostate cancer, and another 2 subtypes of which indicated good survival. Furthermore, we further analyzed the relevant clinical information (such as survival time, survival status, TNM, Age, etc) and found that some related information was not randomly distributed, such as survival status (alive or dead) in 4 clusters. In addition, there were significant differences between T and N. This was similar to the phenomenon found in the study by Xiong and colleagues38 in rectal cancer. Therefore, we could also identify molecular subtype clusters associated with prognosis through AS events. These findings enriched our knowledge of the molecular classification of prostate cancer and provided a large number of biomarker candidates and potential targets for the treatment of prostate cancer.

We describe here the most comprehensive and up-to-date study to assess prognostic factors and long-term survival outcomes for prostate cancer by analyzing abnormal AS variants, splicing factor regulatory networks, and related molecular cluster subtypes. Although the prognostic significance of these potential therapeutic targets for prostate cancer still requires further functional and clinical trials to validate the findings, this research enhances the potential of new clinical biomarkers and therapeutic approaches to help implement new treatment strategy for prostate cancer.

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DISCLOSURE
The authors have no conflicts of interest to declare.

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