Identification of AQP2 as a Prognostic Biomarker in Prostate Cancer through TCGA-Based Transcriptomic Analysis

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Abstract

In this research, differentially expressed genes in prostate cancer have been studied through TCGA database with the aim of elucidating on their functional features and predictive value. Differentially expressed genes (DEGs) were obtained using DESeq2 while enrichment analysis using GO and KEGG was used to examine their biological features. Survival analysis was used to determine their predictive value. It was determined that there are 123 differentially expressed genes (5 upregulated, 118 downregulated) in this cancer type. Through the enrichment analysis, it was established that these genes are mainly involved in regulation of peptidase activity, reproduction, and neuroactive ligand-receptor interactions. The survival analysis found that there is a relationship between the levels of AQP2 gene expression and overall survival rate of patients (P = 0.0098). Therefore, AQP2 can be considered as an important marker for prognosis.

Keywords

Prostate Cancer; Differentially Expressed Genes; TCGA; AQP2; Survival Analysis; Bioinformatics.

1. Introduction

Prostate adenocarcinoma (PRAD) is a malignant neoplasm of prostatic gland epithelial origin, known for having a slow development process and a poor outlook [1]. From data obtained through the Global Burden of Disease study, it can be observed that there has been an increase of 12.2% in the age-standardized incidence rate of prostate cancer from 13.69/100,000 to 15.37/100,000 between 1990 and 2019 [2]. Several reports have noted an increasing trend in PRAD deaths in people who are older than 60 years, with the predicted age-standardized PRAD death rate being 5.74/100,000 by 2030 [3].

Prostate-specific antigen is still one of the widely applied clinical markers; however, it has several drawbacks. The symptoms of prostate cancer are not specific and thus there is a high risk of underdiagnosing and misdiagnosing it [4]. Research shows that the PSA test may show a relatively high rate of false positivity since its positive predictive value may be equal to 25%, while 75% of patients would be subjected to unnecessary prostate biopsy [5]. Recently several studies have revealed new serum and urine biomarkers, as well as the role of molecular markers in diagnosing, prognosing, and risk stratifying prostate cancer. The application of those markers along with PSA can provide a higher accuracy in the diagnosis process [6-8]. It is, therefore, essential to identify new molecular markers.

With the development of high-throughput sequencing technology and the analysis of the biological role of genes via gene expression profiling using publicly available data like The Cancer Genome Atlas (TCGA), there has been an emergence of new possibilities to uncover the molecular mechanism of cancers as well as the discovery of new biomarkers. In this study, the TCGA-PRAD dataset was used to conduct the identification of differentially expressed genes

between prostate cancer and normal tissue by conducting differential expression analysis. Functional enrichment analysis was done to investigate the biological roles of the genes, and survival analysis to evaluate their association with the prognosis of the patients.

2. Materials and Methods

2.1. Data Collection

RNA sequencing (RNA-seq) data and corresponding clinical pathological information for prostate adenocarcinoma (TCGA-PRAD project) were downloaded from the Cancer Genome Atlas database (https://portal.gdc.cancer.gov/) via the UCSC Xena platform (https:// xenabrowser. net/). Gene expression levels were quantified in FPKM (count) format (fragments per kilobase of transcript per million mapped reads) and log2-transformed. Gene annotation utilised the GENCODE v36 reference genome. For genes with multiple transcripts, the highest-expressed transcript was retained by ranking based on average expression across all samples to eliminate redundancy. Data were converted back to raw expression levels using the formula expression = $2^{(\log2\_value)} - 1$, yielding the final gene expression matrix for subsequent analysis.

2.2. Screening for DEGs

Samples were categorised into tumour tissue and normal tissue groups based on TCGA sample barcode characteristics. Differential expression analysis of the gene expression matrix was performed using the DESeq2 package (version 1.50.2, Bioconductor 3.22). Expression data were rounded, and a DESeqDataSet object was constructed, with sample grouping as the design matrix. Standardisation, dispersion estimation, and differential expression testing based on the negative binomial distribution were performed using the DESeq function [9]. The results function was employed to extract differential analysis outcomes, comparing expression changes in tumour tissue relative to normal tissue. DEGs were screened using the criterion of an adjusted P-value ($p < 0.05$). Genes with log2FC > 6 were considered upregulated, and those with log2FC < −4 were considered downregulated. Asymmetric thresholds were adopted to capture the distinct magnitude distributions of upregulated and downregulated genes observed in the TCGA-PRAD dataset. Volcano plots visualising the distribution of DEGs were generated using the ggplot2 package (version 4.4.0), with the x-axis representing log2FC and the y-axis representing −log10(adjusted P-value).

2.3. Functional Enrichment Analysis

Functional enrichment analysis was performed on the selected DEGs. Analysis utilised the clusterProfiler package (version 4.18.4, Bioconductor 3.22), first released in 2012 [10]. This package performs enrichment analysis using GO and KEGG across several model organisms [11], enabling comparison of functional profiles under different conditions. The bitr function was used to convert gene symbols to ENTREZ IDs, and the org.Hs.eg.db package (version 3.22.0, Bioconductor 3.22) was used for gene ID mapping. The enrichGO function was used to perform Gene Ontology (GO) enrichment analysis of DEGs, evaluating functional characteristics at three levels: biological process (BP), cellular component (CC), and molecular function (MF). The enrichment analysis significance threshold was set at the adjusted P-value < 0.05.

KEGG pathway enrichment analysis was performed on DEGs using the enrichKEGG function. The species was defined as ‘hsa’ (human). For statistical analysis, the cutoff value was 0.05. To improve legibility, the setReadable command was used to transform the ID format of genes in the enrichment results of KEGG into gene symbol format. The enrichment results were plotted using the barplot and dotplot commands and showed the top 10 pathways or functions with significant enrichment scores.

2.4. Survival Analysis

The OS dataset for PRAD patients was obtained from the TCGA database, where the data includes the time of survival and survival status of the patient. The survival data was preprocessed such that the time of survival was expressed in months (survival time/30), and samples with no survival time, survival time ≤ 1 month, and no survival status were removed. The gene expression data was then mapped to the survival dataset.

The genes chosen for survival analysis were the top 5 up-regulated and the top 10 down-regulated genes in terms of absolute log2FC value. The optimal cutoff value of gene expression was selected through the 'surv_cutpoint' function from the survminer package (version 0.5.1). This function finds an optimal grouping point of gene expressions based on maximization of survival difference between groups. Based on the cutoff value, patients were divided into two groups according to their gene expressions (high versus low expression). Survival analysis was conducted by Kaplan-Meier method using survival package (version 3.8.3), where survival probability at each time point is calculated through a product of consecutive survival probabilities [12]. Differences in survival curves between two groups were evaluated by the log-rank test, which compares the number of observed and expected events during the follow-up period. The plots of Kaplan-Meier survival curves were drawn using the ggsurvplot function along with P-values.

3. Results

3.1. DEGs Screening Results

Differential expression analysis found 39,043 genes in total out of which 123 were DEGs, including five upregulated and 118 downregulated genes. Moreover, 38,920 genes showed no expression differences (Figure 1). The volcano plot suggests that DEGs are mostly found in the downregulated area. Downregulated genes showed a log2FC from −10 to −4, indicating considerable reduction in expression; whereas upregulated genes showed a log2FC from 6 to 10, but with fewer numbers.

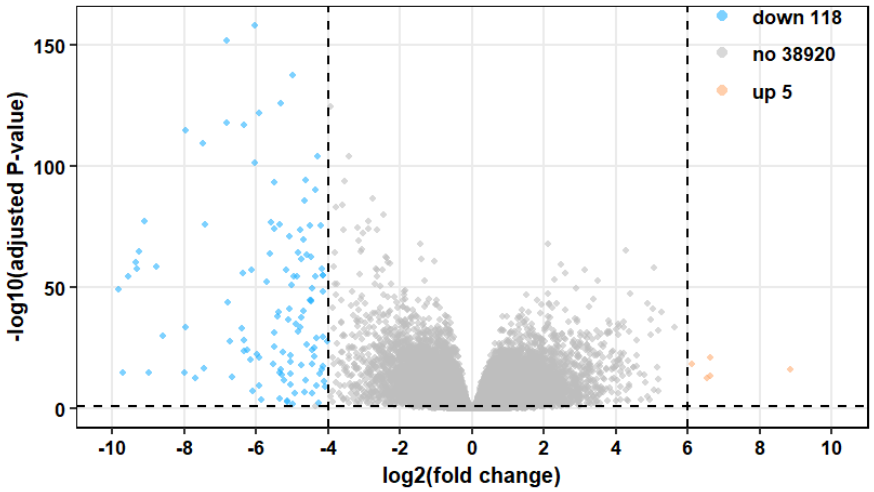

Figure 1. Volcano plot of DEGs.

3.2. Functional Enrichment Analysis of DEGs

3.2.1. Gene Ontology (GO) Functional Enrichment Analysis

GO functional enrichment analysis was performed on 123 DEGs, with the top 10 entries exhibiting the highest significance displayed (Figure 2). Results indicate that DEGs are

predominantly enriched in reproductive processes and in proteolytic regulation. Among these, regulation of peptidase activity emerged as the most significantly enriched term ($P < 0.005$), suggesting peptidase activity plays a significant role in prostate cancer. Cellular processes involved in reproduction in multicellular organisms were also significantly enriched ($P < 0.01$), indicating that the DEGs are closely associated with reproductive system functions.

Other significantly enriched biological processes include: negative regulation of peptidase activity, regulation of reproductive processes, negative regulation of hydrolase activity, negative regulation of proteolysis, single fertilisation, sperm capacitation, and insemination ($P < 0.05$).

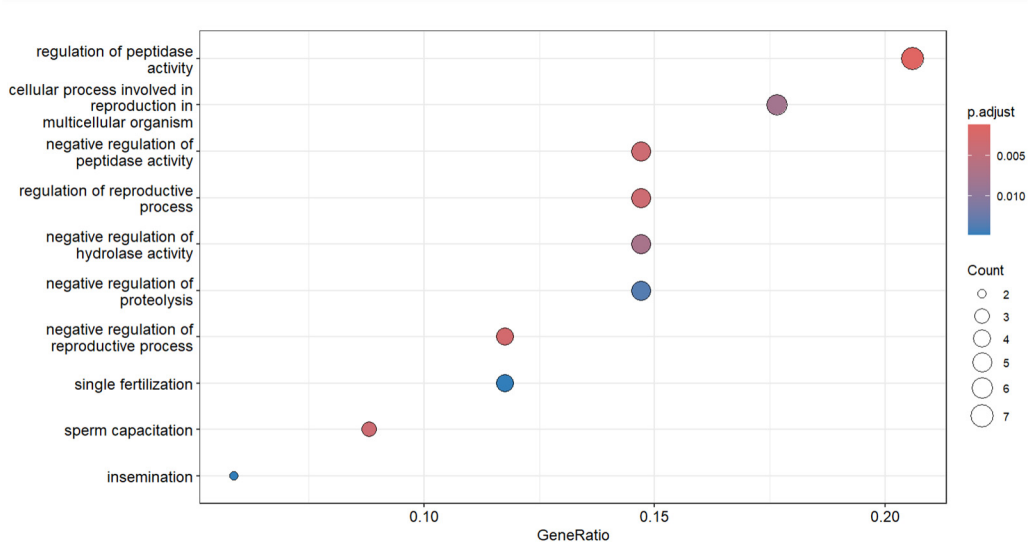

Figure 2. GO enrichment analysis of DEGs.

3.2.2. KEGG Pathway Enrichment Analysis

From KEGG pathway enrichment analysis, it was found that there was significant enrichment of the differentially expressed genes in four pathways (see Figure 3). Of all the four pathways mentioned, the one which showed the highest level of enrichment was the Neuroactive ligand-receptor interaction pathway ($P < 0.015$). The Neuroactive ligand-receptor interaction pathway is known to include interactions among several neurotransmitters and hormones along with their receptors. Thus, there may be changes in neuroendocrine regulation in prostate cancer.

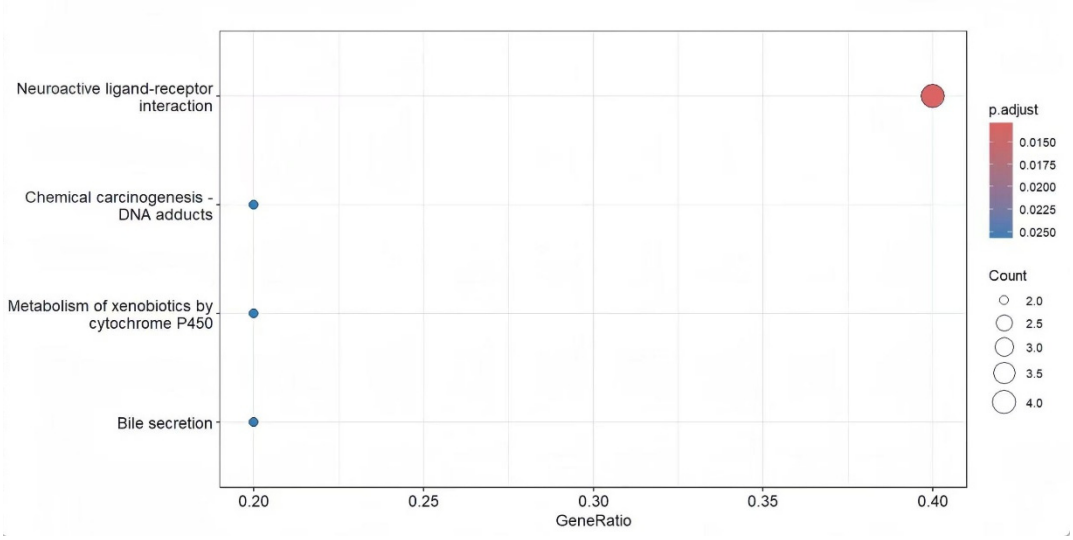

Figure 3. KEGG enrichment analysis of DEGs.

3.3. Relationship between DEGs and Prognosis in Prostate Cancer Patients

To examine the association between DEGs and the prognosis of prostate cancer patients, the top 10 downregulated genes with the smallest log2FC values were chosen (EDDM3A, SEMG1, EDDM3B, SEMG2, AQP2, PAEP, PATE4, TMEM114, DEFB131A, CRISP1). The top five most highly upregulated genes (AC244102.3, AL157387.1, GLYATL3, LINC00993, Z82210.1) were included in the survival analysis.

3.3.1. Survival Analysis of Downregulated Genes

Analysis of survival of down-regulated genes showed a strong correlation between the expression level of AQP2 and the overall survival of patients (P = 0.0098). This shows that the patients with lower expression levels have much higher overall survival rates than those with higher levels, suggesting that downregulation of AQP2 could predict favorable prognosis in prostate cancer patients. TMEM114 gene expression exhibited a marginally significant association with patient prognosis (P = 0.065) (Figure 4), with a trend towards lower expression correlating with better survival outcomes. This did not attain statistical significance.

The expression levels of the remaining six downregulated genes (SEMG1, EDDM3A, PAEP, SEMG2, CRISP1, PATE4) showed no significant correlation with patient overall survival (P > 0.05). Among the ten downregulated genes included in the analysis, survival analysis results for DEFB131A and EDDM3B could not be obtained due to limitations in data distribution.

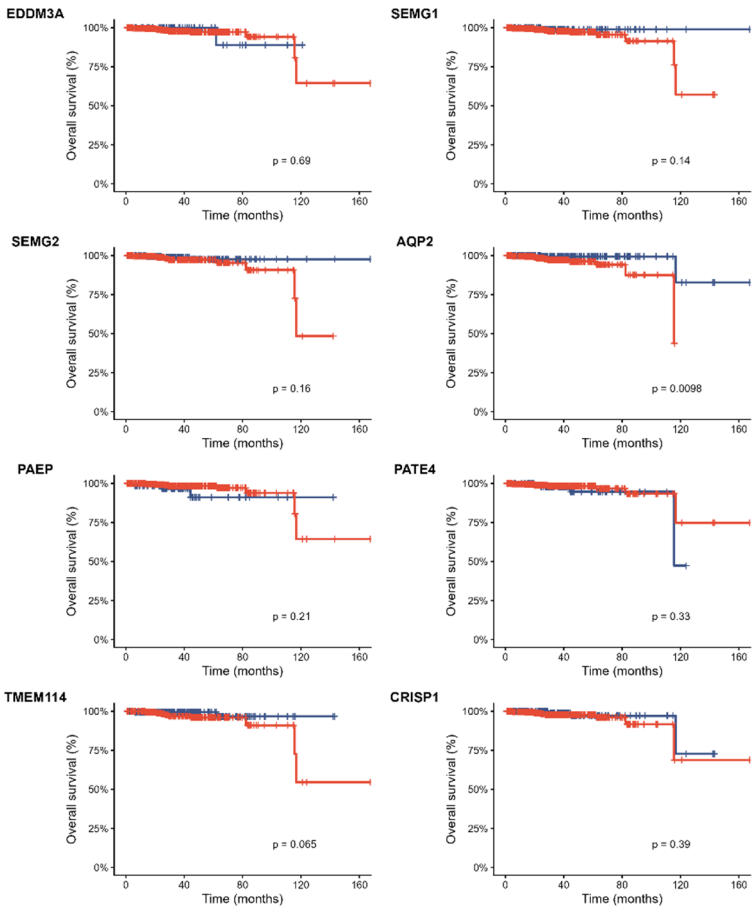

Figure 4. Kaplan-Meier survival analysis of downregulated genes. Red curves represent the high-expression group. Blue curves represent the low-expression group.

3.3.2. Survival Analysis of Upregulated Genes

The survival analysis for the five upregulated genes showed that there was no significant relationship between the levels of the expression of these genes and the overall survival of the patients (P > 0.05) (Figure 5). Z82210.1 had one of the lowest P-values (P = 0.17). AL157387.1 (P = 0.3), AC244102.3 (P = 0.37), GLYATL3 (P = 0.39), and LINC00993 (P = 0.4) exhibited no discernible relationship with patient prognosis.

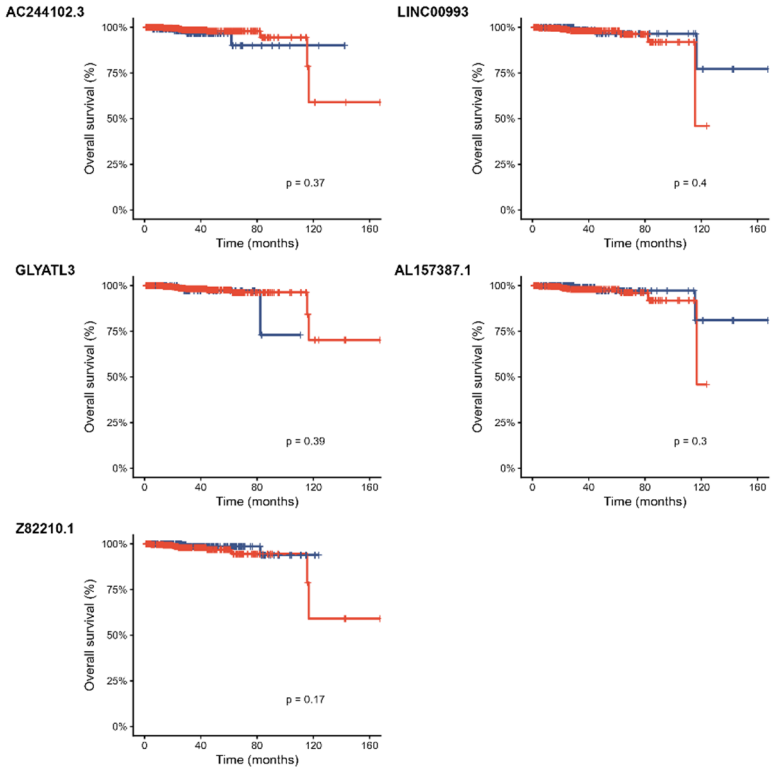

Figure 5. Kaplan-Meier survival analysis of upregulated genes. Red curves represent the high-expression group. Blue curves represent the low-expression group.

4. Discussion

In this research, 123 DEGs were found in TCGA-PRAD database data, with 118 down-regulated genes and 5 up-regulated genes. It implies that in prostate cancer tissue, extensive gene down-regulation is found during malignant transformation. Although several reports about DEGs in prostate cancer are available, Albarracín-Navas et al. [13] conducted an extensive review of 144 differentially expressed mRNAs. There is a general tendency among the existing literature to identify individual candidate genes, rather than systematic analysis of gene expression patterns. The mostly down-regulated pattern of expression observed in this research may be due to extensive repression of normal prostate tissue-specific gene expression during malignant transformation of prostate cancer. This finding is consistent with GO enrichment analysis results, where most of the down-regulated genes were found enriched in secretion and metabolism pathways.

The GO-based functional enrichment analysis demonstrated that the top significant enrichment terms for DEGs included peptidase activity regulation (P < 0.005) and reproductive processes in multicellular organisms (P < 0.01). The latter included several related GO terms such as proteolysis down-regulation, peptidase activity down-regulation, and hydrolase activity down-regulation (P < 0.05, q < 0.05), thus reflecting the dysregulation of the peptidase activity as the key molecular mechanism involved in the pathogenesis of

prostate cancer. Several studies have demonstrated the pivotal role of MMPs in prostate cancer invasion and metastasis, with MMP2, MMP7, and MMP9 being highly expressed in prostate cancer tissue samples and positively correlated with the invasiveness of tumor cells and Gleason score [14]. The upregulated expression of secreted leukocystatin – a protease inhibitor – is also observed in prostate cancer patients and can be regarded as an independent predictor of recurrence and progression of the disease [15].

Enrichment of the reproductive-related functions, which include reproduction process regulation, negative regulation of reproduction processes, single fertilization, sperm capacitation, and fertilization is consistent with the tissue-specificity of prostate as part of the male reproductive organ. The enrichment of tissue-specific genes could be linked to the expression profile of the prostate tumor initiating cells [16]. Canacci et al. observed upregulation of SEMG1 and SEMG2 at the protein level in prostate cancer tissues, with SEMG2 expression negatively correlated with Gleason score [17]. Our study observed significant downregulation of both SEMG1 and SEMG2 at the transcriptomic level, with no significant correlation between their expression levels and patient overall survival. This discrepancy between mRNA and protein expression may relate to post-translational regulation or tumour heterogeneity [18, 19]. The differential expression of these genes may be associated with alterations in the prostate's normal physiological functions.

KEGG pathway enrichment analysis identified four pathways that were significantly enriched. The neuroactive ligand-receptor interaction pathway showed the most significant enrichment ($P < 0.015$), suggesting potential alterations in neuroendocrine signalling within prostate cancer. Research indicates that prostate neuroendocrine cells produce multiple neuroactive substances, with these neuroactive ligand-receptor interactions playing a role in prostate cancer progression [20]. Puca et al. [21] highlighted that abnormal activation of multiple neuroactive ligands and their corresponding receptors constitutes a key mechanism driving tumour progression during the transdifferentiation of castration-resistant prostate cancer towards a neuroendocrine phenotype. The enrichment of the chemocarcinogen-DNA adducts and cytochrome P450-mediated exogenous substance metabolism pathway ($P < 0.05$) suggests that environmental factors and metabolic processes may contribute to the initiation and progression of prostate cancer. Apart from participating in the activation of exogenous carcinogens to produce DNA adducts, the cytochrome P450 enzyme system is also involved in the metabolism of oestrogens and androgens leading to the production of carcinogens resulting in prostate cancer development [22]. In another study by Xiao et al., the presence of DNA adducts of PhIP, a carcinogen found in cooked meat, was detected in 31% of prostate cancer patient tissues indicating a link between dietary carcinogens and prostate cancer [23]. There was a tendency for the pathway of bile secretion to be enriched in this study. It is known that the prostate is not part of the digestive system. However, recent studies indicate the possible role of bile acids and their receptors such as FXR, VDR in the regulation of prostate cancer.

Overall survival was significantly higher in individuals having lower AQP2 expression than in patients with higher expression levels ($P = 0.0098$). Since AQP2 is a member of the aquaporin family which is activated by vasopressin, AQP2 is found mostly in renal collecting ducts and controls water reabsorption and urine concentration [25]. Furthermore, AQP2 expression was found in fallopian tubes and pancreatic islets and is also secreted through exosomes in the urine [26]. The function of AQP2 is influenced by different PTMs. AQP2 is phosphorylated through cAMP/PKA signaling in response to vasopressin and leads to apical membrane localisation [27]. Besides phosphorylation, AQP2 is also affected by S-glutathionylation [28]. New reports show that AQP2 acts as a non-invasive biomarker for prostate cancer. A risk model of H4C2 and ZNF114 had excellent diagnostic and prognostic performance (AUC = 0.965) while AQP2 has diagnostic AUC = 0.736–0.809 [29]. AQP2 is found to be

underexpressed unlike other aquaporins [30], but its expression level is correlated with prostate cancer prognosis.

In a study by Qiu et al. [31] in which TGF-β1 was used to induce fibrosis in bladder urothelial cells, it was found that AQP2 down-regulation facilitated EMT, proliferation, and migration but up-regulation of AQP2 caused reversal of these phenomena. It was further seen that the transcription factor FOXO1 regulates the expression of AQP2, hence indicating that the disturbance in the FOXO1/AQP2 signalling pathway could be one of the important molecular events in the development of prostate cancer [32]. In a comprehensive study conducted by Bründl et al. [33], all 13 AQPs were screened in prostate tissues, and it was found that there is no expression of AQP2, but the expression of AQP3 decreases with the increase in malignancy of the tumour. There was a marginally significant difference for the gene TMEM114 ($P = 0.065$).

Expression level of the other eight down-regulated genes (SEMG1, EDDM3A, PAEP, SEMG2, CRISP1, PATE4 and two others) had no significant correlation with the overall survival of the patients ($P > 0.05$). There was no significant relationship between the expression level of the five up-regulated genes and the overall survival of the patients ($P > 0.05$). The Z82210.1 gene exhibited a relatively low P-value ($P = 0.17$), whilst the remaining genes AL157387.1 ($P = 0.3$), AC244102.3 ($P = 0.37$), GLYATL3 ($P = 0.39$), and LINC00993 ($P = 0.4$) demonstrated higher P-values. This result implies that despite being upregulated in the prostate cancer tissues at high log2FC values, the expression of these five genes does not affect the overall survival of patients. It is consistent with one of the frequently observed phenomena in the field of prostate cancer where not all DEGs are correlated with the prognosis of patients. In their study, He et al. performed a comprehensive analysis of both GEO and TCGA databases and selected 10 hub genes out of 484 DEGs. It was shown that only the mutations of two genes, namely ITGB4 and RRM2, were significantly related to the survival of patients ($P<0.01$) [34]. In their large-scale analysis of the TCGA database, Myers et al. found 11,115 DEGs but only certain signalling pathways were identified as significantly related to the development of prostate cancer [35].

5. Conclusion

Detailed analysis indicates that the molecular features of prostate cancer mainly include large-scale down-regulation of genes. The identified DEGs mainly involved functions related to regulation of peptidase activity, reproduction, neuroendocrine signal transduction, and immunity. As for the prognosis, only AQP2 was found to have prognostic significance, as the high expression level of this gene was significantly associated with poor prognosis in prostate cancer patients, implying that AQP2 can be considered as a promising prognostic marker for prostate cancer. Most DEGs, especially the up-regulated ones, were not correlated with prognosis.

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