Original Research

GAS1RR, an immune-related enhancer RNA, is related to biochemical recurrence-free survival in prostate cancer

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Impact Statement

Enhancer RNA (eRNA) has been increasing identified as a key regulator in pathologies including cancer. However, the role of eRNA in prostate cancer (PCa) remains largely unknown. Our data revealed that GAS1RR is highly expressed in normal prostate tissues; low expression of GAS1RR is related to shorter time to biochemical recurrence of PCa and is relevant to many clinical pathological traits. Multivariate analysis indicates that it has an independent prognostic value in PCa. Functional pathway analysis revealed that cell adhesion and extracelluar matrix–related pathways may take part in the development of PCa. Research on mechanism in the future will further illuminate the function of GAS1RR in PCa.

Abstract

Prostate cancer (PCa) is one of the malignant tumors of urinary system with a high morbidity. Enhancer RNA is a subclass of long non-coding RNA transcribed from active enhancer regions, which plays a critical role in gene transcriptional regulation. However, the role of enhancer RNA (eRNA) in PCa remains extremely mysterious. This study is aimed at exploring key prognostic eRNAs in PCa. First, we downloaded gene expression data and clinical data of 33 cancer types from UCSC Xena platform. Second, we selected reported putative eRNA-target pairs and performed the Kaplan–Meier survival and correlation analysis to determine the crucial eRNAs most related to biochemical recurrence (BCR)-free survival. Third, we explored the clinical characteristics with the key eRNA GAS1 adjacent regulatory RNA (GAS1RR) and performed a computational difference algorithm and the Cox regression analysis. Next, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to explore the underlying mechanisms. Finally, we used the pan-cancer data from The Cancer Genome Atlas (TCGA) and performed reverse transcription-quantitative polymerase chain reaction (RT-qPCR) of 18 pairs of specimens to prove the results we acquired. Among all 2695 putative eRNAs, 6 pairs of eRNA-target genes were prominently related to BCR-free survival. Growth arrest-specific protein 1 (*GAS1*) was a target gene of GAS1RR ($r = 0.86$, $P < 0.001$). Patients with low GAS1RR expression were likely to have unfavorable clinical characteristics. The result of computational Cox regression analysis demonstrated that GAS1RR may predict the prognosis of PCa independently. RT-qPCR results illuminated that GAS1RR and GAS1 were both downregulated in PCa tissues, and they show a strong positive correlation. GO and KEGG analyses revealed biological processes that GAS1RR was mainly associated with. Immune infiltration analysis indicated that GAS1RR expression is correlated with the infiltration level of six kinds of immune cells. Our results suggest that GAS1RR may be clinically useful in the prediction of PCa prognosis. Moreover, it may also be a prognostic predictor and theoretic target with great promise in PCa.

Keywords: Prostate cancer, prognosis, biochemical recurrence, enhancer RNA, immune infiltration, GAS1RR

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Introduction

Prostate cancer (PCa) is one of the most frequently diagnosed cancer types in many industrialized developed countries, and its morbidity is ascending all around the world. Furthermore, the incidence of PCa ranked first in all the male malignant tumors. Of all incident cases in men in 2021, PCa alone accounted for 26% of diagnoses,[1] with the reality that there were approximately 248,530 new cases as well as 34,130 people dead in 2021 in the United States. Therapeutic regimens nowadays incorporating radical prostatectomy (RP) and radical radiation therapy (RT) have become a publicly accepted treatment choice for localized PCa. Whereas, nearly 20–40% of PCa patients may undergo biochemical recurrence (BCR).[2,3] BCR is conventionally defined as the reverse of declining prostate-specific antigen (PSA) value, a growing amount of evidence illustrates that PCa patients who have BCR after RT or RP possess an increasing risk of latter PCa-specific mortality and are more likely to metastasize without effective interference. Therefore, it is urgently

needed to identify relative biomarkers to discern patients with high-stake of BCR.[4,5]

Enhancer RNA (eRNA) is a novel subclass of non-coding RNA, they are usually generated from DNA regions containing activated enhancer.[6,7] Accumulating evidence has indicated that eRNAs have important functions in cancer and disease development through their regulation of transcription, RNA processing, and translation.[7–10] However, few studies have paid attention to the role of eRNA in the field of urology, especially in PCa. In this research, we probed into and ascertained eRNAs relevant to BCR-free survival in PCa patients. GAS1 (Growth Arrest-Specific Protein 1) is a tumor suppressor validated in a large number of published papers, it appears that the GAS1 protein is an integral plasma membrane protein, and its expression is linked to growth cessation. When GAS1 is overexpressed from a constitutive promoter in quiescent cells, the serum-induced cell cycle transition from the G0 to the S phase of the cell cycle is inhibited without affecting the normal early serum res ponse,[11–17] and we discovered the eRNA GAS1RR (GAS1 Adjacent Regulatory RNA), located nearby its putative target GAS1, was observably interrelated with beneficial clinical and pathological features and longer time to BCR in PCa patients. In addition, by analyzing the data from Tumor Immune Estimation Resource (TIMER) database, we found GAS1RR may influence the proportion of immune cells. Therefore, it has the potential to become a therapeutic target and prognostic predictor. We present the following article in accordance with the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) reporting checklist.

Materials and methods

Acquisition of public available data and eRNAs of prognostic value in PCa

First, we downloaded the gene expression profiles and pan-cancer data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/repository) as well as corresponding clinical data from the UCSC Xena (https://xenabrowser.net/datapages/) database. Then, the expression data and clinical data were matched, so that only patients possessing clinical information and expression profiles were filtered to be enrolled in further research. Second, we acquired a list of eRNAs transcribed from active enhancers regions and their predicted target genes by making use of the Predicting Specific Tissue Interactions of Genes and Enhancers (PreSTIGE) method.[18] Finally, we use the R packages "survival" and "survminer" to explore the relevance between the expression profile of predicted eRNAs and BCR-free survival of PCa patients.

Differential gene expression analysis

After getting the expression data of 33 kinds of cancers, we analyzed the RNA-seq data of GAS1RR and GAS1 between normal and tumor samples in different cancer types using "edgeR" package. Ultimately, when $|\log2foldchange| \geqslant 1$ and $P < 0.05$, it was regarded significantly different between tumor and normal samples.

Co-expression analysis

According to the expression profile of predicted eRNAs and their putative target genes, we performed the co-expression analysis between them. In the result, eRNAs which live up to the criterion listed below: significant association with BCR-free survival (Kaplan–Meier log rank of $P < 0.05$) and co-expression with the predicted targets ($r > 0.3$ and $P < 0.001$), they will be considered as candidates for the next analysis. Then, we chose the eRNA which had the closest connection with BCR to conduct further research. The expression profile of eRNA and its target were also investigated by analyzing their respective expression level in PCa tissues and normal prostate specimens. Moreover, the correlation was also verified in 32 kinds of other cancers.

Independent prognostic analysis of GAS1RR

We investigated the independent prognostic value of eRNA in PCa. Univariate and multivariate analyses were applied to explore whether GAS1RR could independently predict BCR-free survival in PCa patients in the TCGA cohorts.

Functional enrichment analysis

For the purpose of investigating the potential mechanism of GAS1RR, we conducted Spearman's correlation analysis using the gene expression data from the UCSC Xena database to identify the co-expressed genes of GAS1RR in PCa. Subsequently, 4538 co-expressed target genes (Spearman correlation coefficient $r > 0.3$ and $P < 0.001$) of GAS1RR were selected to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis by "clusterProfiler" package.

Immune estimation of GAS1RR and GAS1

We made use of immune data downloaded from the TIMER database (version 2.0) to evaluate the expression of eRNA GAS1RR and its target gene *GAS1* and their relationship with immune infiltration abundance by R software,[19,20] and six types of important immune cells were enrolled in the analysis.

Extraction of RNA and reverse transcription-quantitative polymerase chain reaction

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was conducted to further verify RNA-seq data downloaded from TCGA. A total of 18 pairs of PCa samples and adjacent normal specimens from surgical patients undergoing RP in Tongji Hospital from 2020 to 2021 were collected by us. All patients participating in the study fully understood and gave informed consent. To quantify the expression of GAS1 and GAS1RR, total RNA of PCa and normal tissues was extracted through the use of TRIzol, and complementary DNA (cDNA) was synthesized using cDNA synthesis kit under the guidance of the manufacturer's protocol. Quantitative PCR was performed using the SYBR Green Master mixture using ABI QuantStudio 6 RT-qPCR instrument (USA). We adopt glyceraldehyde

Table 1. eRNAs relevant with BCR-free survival and their putative target gene.

eRNA	Log-rank test *P* value	Predicted target	Correlation coefficient *r*	Spearman *P* value
GAS1RR	0.007	GAS1	0.862	5.36e−149
AP002992.1	0.006	CHKA	0.472	2.15e−29
AP001208.1	<0.001	GRHL2	0.528	3.67e−37
PCBP1-AS1	0.001	TIA1	0.676	4.06e−68
ADCY10P1	0.029	UNC5CL	0.665	3.24e−65
PCBP1-AS1	0.001	ASPRV1	0.409	8.42e−22

eRNA: enhancer RNA; BCR: biochemical recurrence; GAS1RR: GAS1 adjacent regulatory RNA.

3-phosphate dehydrogenase (GAPDH) as the internal standard. The primers of GAS1RR and its target GAS1 were synthesized by Qingke Biological Technology Co., Ltd. (China). The primers sequences were as follows: GAS1RR (forward: AAGGAAAGAGATGCCTGGCC; reverse: GGCAACCAAGGTAAGCTCCT), GAS1 (forward: ATGCCGCACCGTCATTGAG; reverse: TCATCGTAGTAGTCGTCCAGG), and GAPDH (forward: ACAACTTTGGTATCGTGGAAGG; reverse: GCCATCACGCCACAGTTTC). Ultimately, we used $2^{-\Delta\Delta Ct}$ method to normalize the gene expression levels between different groups.

Statistical analysis

We processed and analyzed all the data by statistical software R (version 4.1.0), mainly using the packages of Bioconductor (http://www.bioconductor.org/). And gene expression level between two groups was compared by variance analysis or *t*-test. The differential expression of GAS1RR or GAS1 was evaluated between 18 pairs of PCa and normal specimens by paired Student's *t*-test. For the purpose of assessing the correlation strength between GAS1RR and GAS1, we used Spearman's rank correlation coefficient as a measure. The result with P value < 0.05 was regarded as a significant result, unless stated otherwise.

Results

Putative prognostic eRNAs in PCa

Patients with PCa were enrolled in this study after their gene expression data were combined with clinical information. Among them, patients with BCR status information were selected for further analysis. Then, eRNAs and their putative targets were determined from the ENCODE database by PreSTIGE algorithm. As a result, a total of 2695 eRNAs produced from active enhancers were determined.[18] These eRNA-target pairs were used to identify potential key eRNAs in PCa. Finally, six pairs were authenticated with the following conditions: (1) Kaplan–Meier log rank of $P < 0.05$ and (2) correlation coefficient $r > 0.3$ and $P < 0.001$ (Table 1). It turned out that GAS1RR, AP002992.1, AP001208.1, PCBP1-AS1, ADCY10P1, and PCBP1-AS1 were associated with BCR-free survival. Besides, the flow chart of this study is shown in Figure 1.

GAS1RR is identified as a pivotal eRNA in PCa

As is represented in Table 1, six pairs of eRNAs and their targets are relevant with BCR-free survival, we sorted them by the correlation coefficients, and the strongest correlation was found between GAS1RR and GAS1. Therefore, we executed further analysis for GAS1RR. On the basis of the expression data of 499 PCa specimens and 52 normal prostate specimens, we found that GAS1RR and its target GAS1 expression were significantly higher in normal prostate specimens compared to malignant ones ($P < 2.22e{-}16$) (Figure 2(a)). Next, on the basis of the GAS1RR expression level, individuals with BCR information were separated into high-GAS1RR and low-GAS1RR expression groups, respectively. Then, we performed Kaplan–Meier survival analysis and found that low expression of GAS1RR was significantly associated with shorter time to BCR ($P < 0.0001$, Figure 2(b)). Furthermore, by performing co-expression analysis, we found out that there existed a powerful relation between GAS1RR and GAS1 (Figure 2(c), $r = 0.86$, $P < 2.2e{-}16$). Aiming to verify the aforementioned results, we explored the differential expression and prognostic value of GAS1RR and its correlation with GAS1 in pan-cancer data (32 other cancer types) from TCGA database as an internal validation. It is worth noting that GAS1RR expression was markedly lower in many other types of cancers than in normal tissues, including bladder urothelial carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma, and endocervical adenocarcinoma and many other types of cancer (Figure 2(d)). GAS1RR also had a prognostic function in adrenocortical carcinoma, bladder urothelial carcinoma, breast invasive carcinoma (Table 3), and so on. Interestingly, GAS1RR and GAS1 also showed significant correlations in other 32 kinds of cancers (Table 3 and Supplemental Figure S1). These results indicate that GAS1RR may inhibit the progress of PCa by rising the expression of target gene *GAS1*; therefore, it is selected as a pivotal eRNA in PCa.

GAS1RR expression is notably related to a variety of clinical features of PCa

We next explored the relationship between GAS1RR expression and clinicopathological features of PCa. Supplemental Table S1 listed some important clinical traits of PCa patients getting from TCGA data set, including age, Gleason score, pathologic T, PSA value, cancer status, and biochemical recurrence. As is shown in Figure 3(a), patients with low Gleason score had higher expression of GAS1RR than individuals with higher score ($P = 0.00058$). As for PSA value, patients with higher PSA value tend to have lower GAS1RR expression ($P = 0.014$, Figure 3(b)). In Figure 3(c), it turned out that pathologic T stage had an inverse correlation with the expression of GAS1RR ($P = 0.0017$).

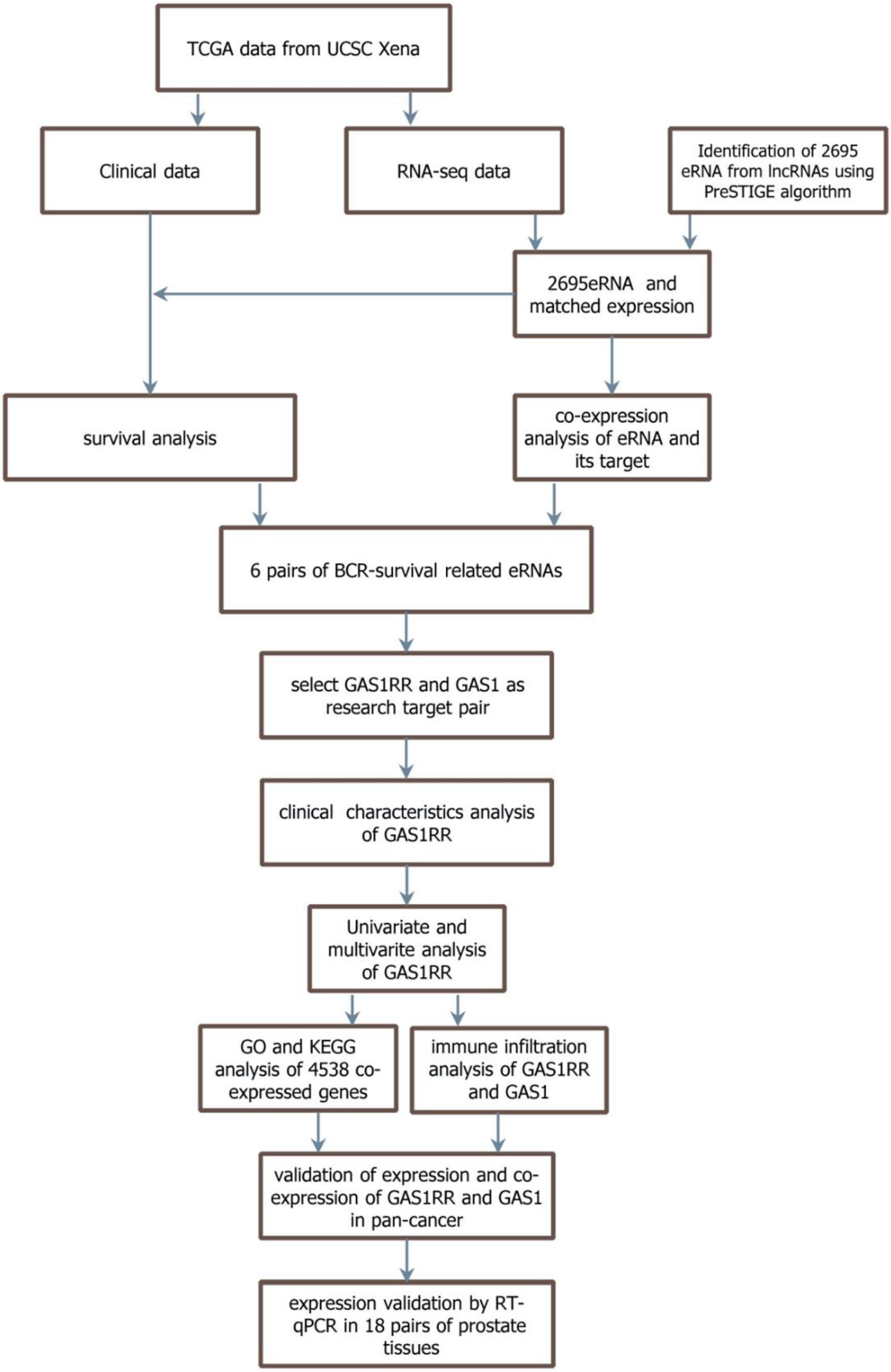

Figure 1. The flow chart of this article.
GO: Gene Ontology; KEGG: Enriched Kyoto Encyclopedia of Genes and Genomes; RT-qPCR: reverse transcription-polymerase chain reaction.

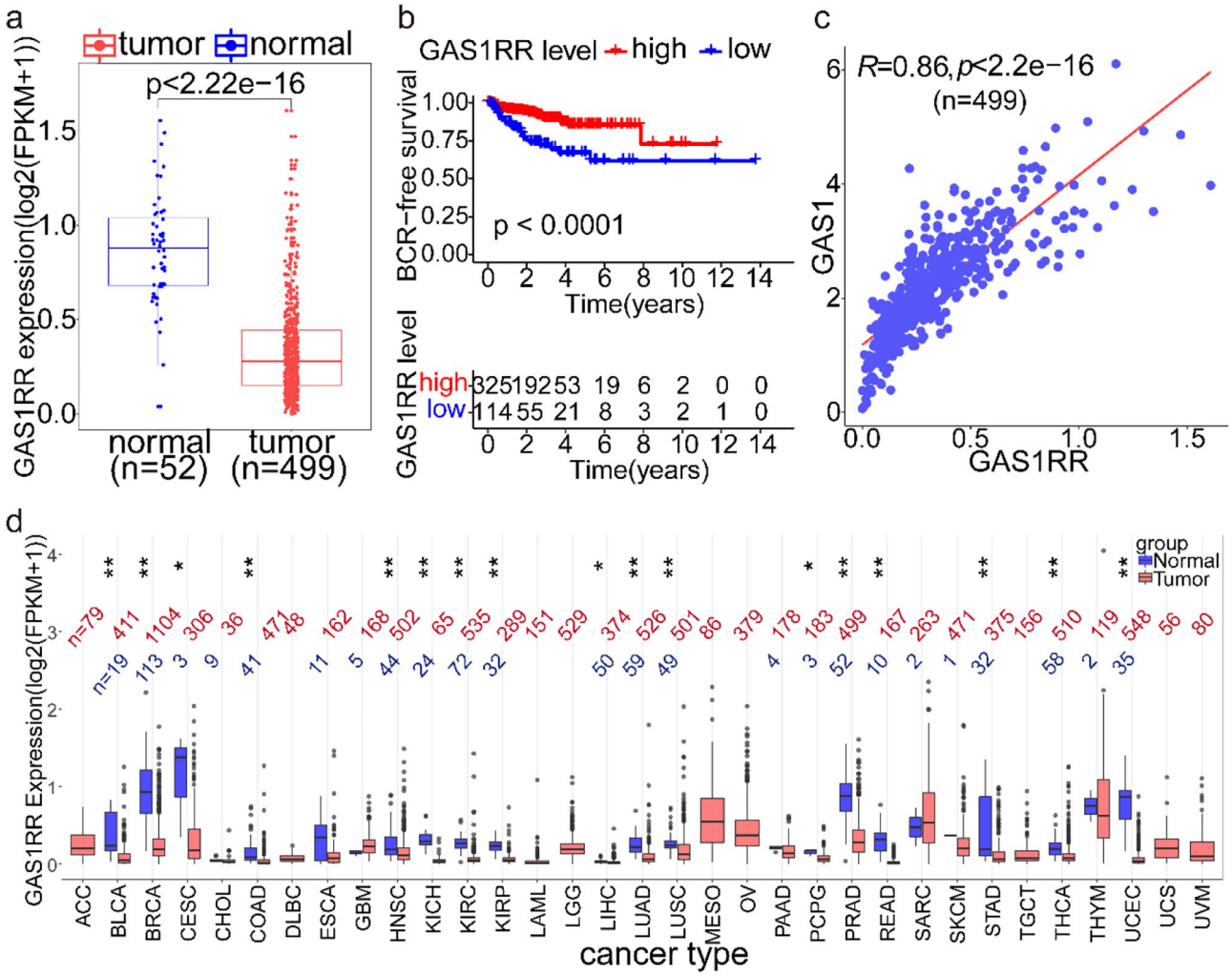

Figure 2. GAS1RR is a vital eRNA of PCa. (a) GAS1RR expression is significantly lower in PCa tissues when compared with normal ones; (b) Kaplan–Meier survival in high GAS1RR and low GAS1RR expression groups among TCGA cohorts; (c) GAS1RR has a strong positive correlation with its putative target GAS1; and (d) expression profiles of GAS1RR in 33 types of cancers from UCSC data set.

However, there existed no obvious discrepancy in GAS1RR expression in the two age groups with a threshold of 55 years ($P = 0.081$; Figure 3(d)). In Figure 3(e), it was manifested that patients bearing tumor appeared to have lower GAS1RR expression ($P = 0.014$). In Figure 3(f), patients with BCR had markedly lower GAS1RR expression ($P = 0.0033$). Finally, using the median expression of GAS1RR as a baseline, patients were separated into two risk group. Chi-square test indicated that GAS1RR expression was apparently correlated to Gleason score, pathologic T, PSA value, cancer status, and biochemical recurrence (Table 2). These results together indicated that GAS1RR expression was closely relevant to the severity of PCa.

Value of GAS1RR in independent prognostic prediction of PCa

As survival analysis pointed out that GAS1RR expression level was related to BCR-free survival of PCa patients, we next thought about whether GAS1RR could be an independent prognostic predictor. We conducted univariate and multivariate Cox regression analyses. In consideration of the fact that some information such as T stage, N stage, M stage and some other information were lacking or were not uniformly spread over most cases, these data were eliminated. Finally, patients having information regarding Gleason score, PSA, pathologic T, age, and GAS1RR expression were brought into the analysis. As a result, we discovered the observable difference in high-GAS1RR and low-GAS1RR groups in univariate (Supplemental Table S2, hazard ratio (HR) = 0.165; 95% confidence interval (CI) = 0.042–0.649; $P = 0.010$) and multivariate (Supplemental Table S3, HR = 0.188; 95% CI = 0.042–0.834; $P = 0.028$) groups (Figure 4(a) and (b)). The consequence manifested that GAS1RR had an independent value in predicting the prognosis of PCa patients.

Detection of GAS1RR and GAS1 expression quantity using RT-qPCR

RT-qPCR was used to profile the expression of these two genes in 18 confirmed PCa patients. As compared with the corresponding normal tissues, the expression of GAS1RR

Table 2. Correlations between the level of GAS1RR/GAS1 expression and clinicopathological traits in PCa.

Characteristic	*n* (%)	GAS1RR expression		*P* value
		High	Low	
Total	499 (100)	249	250	
Gleason score				0.005
⩽7	293 (58.72)	162	131	
>7	206 (41.28)	87	119	
PSA value				0.019
⩽10	329 (65.93%)	173	156	
>10	151 (30.26%)	68	83	
Unknown	19 (3.81%)	22	35	
Pathologic T				0.041
T2	189 (39.88)	105	84	
T3–T4	303 (60.72)	139	164	
Unknown	7 (1.40)	5	2	
Age (years)				0.077
⩽55	110 (22.04)	62	48	
>55	389 (77.96)	187	202	
Biochemical recurrence				0.052
No	371 (74.35)	197	174	
Yes	59 (11.82)	24	35	
Unknown	69 (13.83)	28	41	
Cancer status				<0.001
Tumor free	289 (57.92)	166	123	
With tumor	53 (10.62)	20	33	
Unknown	157 (31.46)	63	94	

PSA: prostate-specific antigen; GAS1RR: GAS1 adjacent regulatory RNA; GAS1: growth arrest-specific protein 1.

Table 3. KM survival analysis and correlations analysis for GAS1RR and GAS1 in pan-cancer (32 other types of cancer from TCGA).

Abbreviation	Full name	Log-rank test *P* value	Correlation coefficient	Spearman *P* value
ACC	Adrenocortical carcinoma	0.888	0.543	1.15e−07
BLCA	Bladder urothelial carcinoma	0.345	0.834	8.14e−108
BRCA	Breast invasive carcinoma	0.205	0.728	4.46e−183
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.067	0.783	6.46e−65
CHOL	Cholangio carcinoma	0.755	0.820	4.81e−10
COAD	Colon adenocarcinoma	0.072	0.687	3.03e−67
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma	0.508	0.330	1.10e−02
ESCA	Esophageal carcinoma	0.859	0.753	3.06e−31
GBM	Glioblastoma multiforme	0.170	0.163	1.75e−02
HNSC	Head and neck squamous cell carcinoma	0.097	0.586	6.81e−48
KICH	Kidney chromophobe	0.457	0.707	2.38e−11
KIRC	Kidney renal clear cell carcinoma	0.920	0.611	1.98e−56
KIRP	Kidney renal papillary cell carcinoma	0.433	0.711	3.56e−46
LAML	Acute myeloid leukemia	0.001	0.575	6.08e−15
LGG	Brain lower grade glioma	0.069	0.388	8.36e−21
LIHC	Liver hepatocellular carcinoma	0.001	0.690	1.67e−54
LUAD	Lung adenocarcinoma	0.228	0.687	5.08e−75
LUSC	Lung squamous cell carcinoma	0.001	0.730	8.17e−85
MESO	Mesothelioma	0.045	0.710	1.01e−14
OV	Ovarian serous cystadenocarcinoma	0.541	0.626	6.95e−43
PAAD	Pancreatic adenocarcinoma	0.695	0.616	2.59e−20
PCPG	Pheochromocytoma and paraganglioma	0.4472	0.462	2.28e−11
READ	Rectum adenocarcinoma	0.148	0.649	1.32e−21
SARC	Sarcoma	0.116	0.472	2.88e−16
SKCM	Skin cutaneous melanoma	0.033	0.586	4.59e−45
STAD	Stomach adenocarcinoma	0.204	0.739	2.28e−66
TGCT	Testicular germ cell tumors	0.414	0.752	5.50e−30
THCA	Thyroid carcinoma	0.204	0.586	1.31e−48
THYM	Thymoma	0.100	0.645	1.24e−15
UCEC	Uterine corpus endometrial carcinoma	0.020	0.741	9.91e−97
UCS	Uterine carcinosarcoma	0.411	0.741	3.41e−11
UVM	Uveal melanoma	5.85E−05	0.652	2.78e−11

KM: Kaplan–Meier; GAS1RR: GAS1 adjacent regulatory RNA; GAS1: growth arrest-specific protein 1; TCGA: The Cancer Genome Atlas.

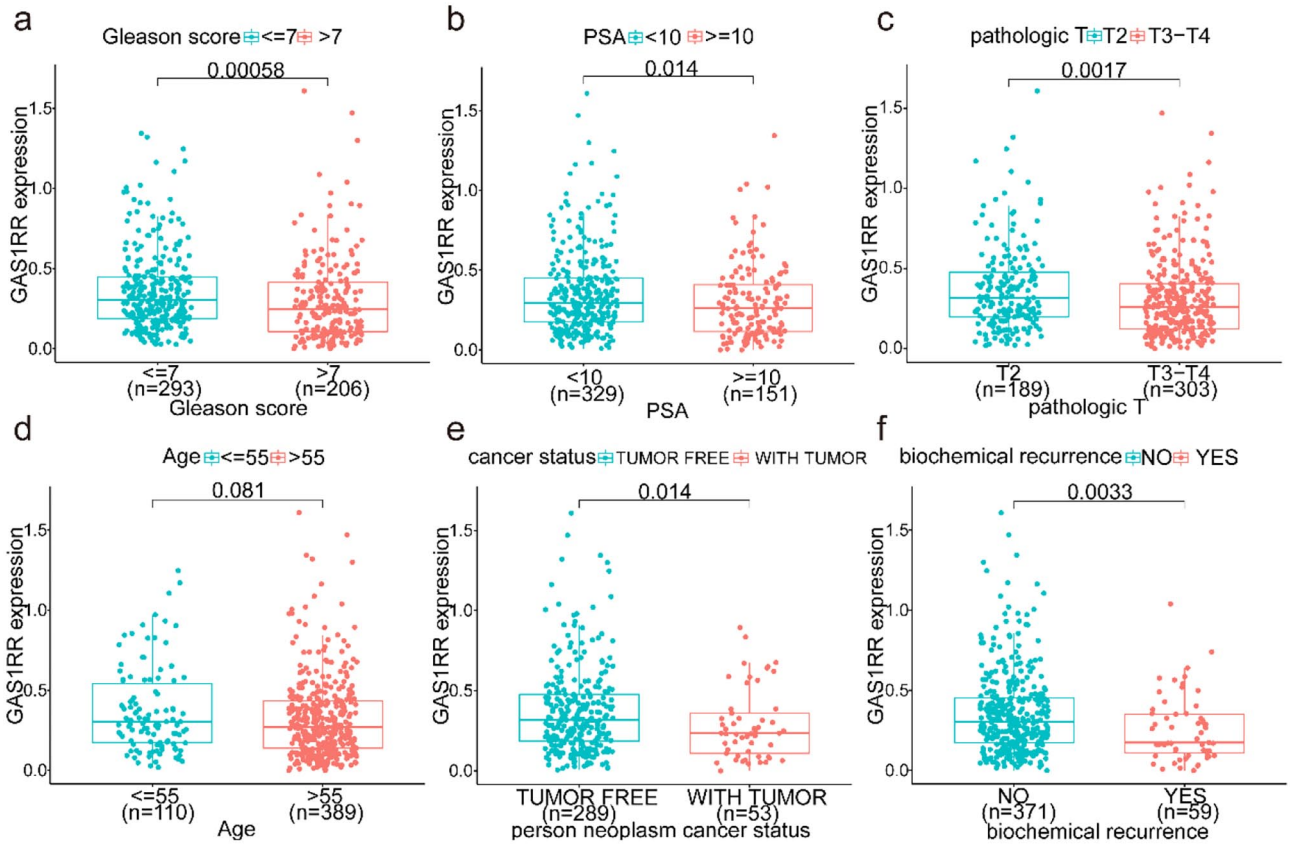

Figure 3. Relationship between the expression of GAS1RR and key clinicopathological traits of PCa. (a) GAS1RR expression significantly decreased with increasing Gleason score, (b) GAS1RR expression significantly decreased with increasing PSA value, (c) GAS1RR expression significantly decreased with advanced pathologic T, (d) GAS1RR expression is not significantly correlated with age, (e) GAS1RR expression significantly decreased with person neoplasm cancer status, and (f) GAS1RR expression significantly decreased with BCR status.

and GAS1 is significantly reduced in malignant tissues (Figure 5(a) and (b)). Furthermore, the results of Spearman's correlation analysis implied that GAS1RR expression showed a positive correlation with GAS1 expression in both tumor samples (Figure 5(c), $r = 0.85$) and normal samples (Figure 5(d), $r = 0.81$).

Functional enrichment analysis and immune infiltration analysis of GAS1RR

We performed GO and KEGG functional enrichment analyses on 4538 genes significantly co-expressed with GAS1RR ($r > 0.3$, $P < 0.001$). The GO functional enrichment analysis results indicated that GAS1RR may function by regulating external structure organization and cell adhesion (Figure 6(a)). KEGG functional enrichment analysis suggested that many cancer-related pathways such as calcium and mitogen-activated protein kinase (MAPK) signaling pathway were enriched (Figure 6(b)), revealing that GAS1RR may function through these pathways in the carcinogenesis of PCa. Interestingly, "Focal adhesion" and "Cell adhesion" were enriched, which were consistent with the result of GO analysis. By use of TIMER, we investigated the relation between GAS1RR, GAS1, and immune infiltration, respectively. It turned out that GAS1RR and its target GAS1 were strongly correlated with six kinds of important immune cells. In Figure 6(c), GAS1RR expression was positively correlated to B cell ($r = 0.25$ and $P = 1.3e{-}08$), CD4+ T cell ($r = 0.45$, $P = 1.1e{-}26$), CD8+ T cell ($r = 0.38$, $P = 7.7e{-}19$), macrophage ($r = 0.54$, $P = 2.7e{-}39$), dendritic cell ($r = 0.38$, $P = 5.6e{-}19$), and neutrophil ($r = 0.45$, $P = 7.0e{-}27$) infiltration, indicating its pivotal roles in immune regulation.

Correlation between GAS1 and BCR-free survival and immune cell infiltration

As expected, the expression of GAS1 was significantly higher in normal tissues compared with PCa tissues (Figure 7(a), $P = 2.8e{-}15$), and the low expression was related to shorter time to BCR (Figure 7(b), $P = 0.003$). Then, we validated the result we acquired in pan-cancer analysis, and a consistent result was shown in many kinds of cancers in Figure 7(c), indicating the role of GAS1 in the survival of PCa patients. Moreover, by TIMER database, we found that the expression level was also distinctly related to the infiltration level of six kinds of immune cells as is shown in Figure 7(d). The result indicated that GAS1 expression was positively correlated to B cell ($r = 0.24$, $P = 2.0e{-}08$), CD4+ T cell ($r = 0.49$, $P = 5.2e{-}31$), CD8+ T cell ($r = 0.38$, $P = 4.0e{-}19$), macrophage ($r = 0.56$, $P = 1.7e{-}42$), dendritic cell ($r = 0.41$, $P = 1.9e{-}21$), and neutrophil ($r = 0.46$,

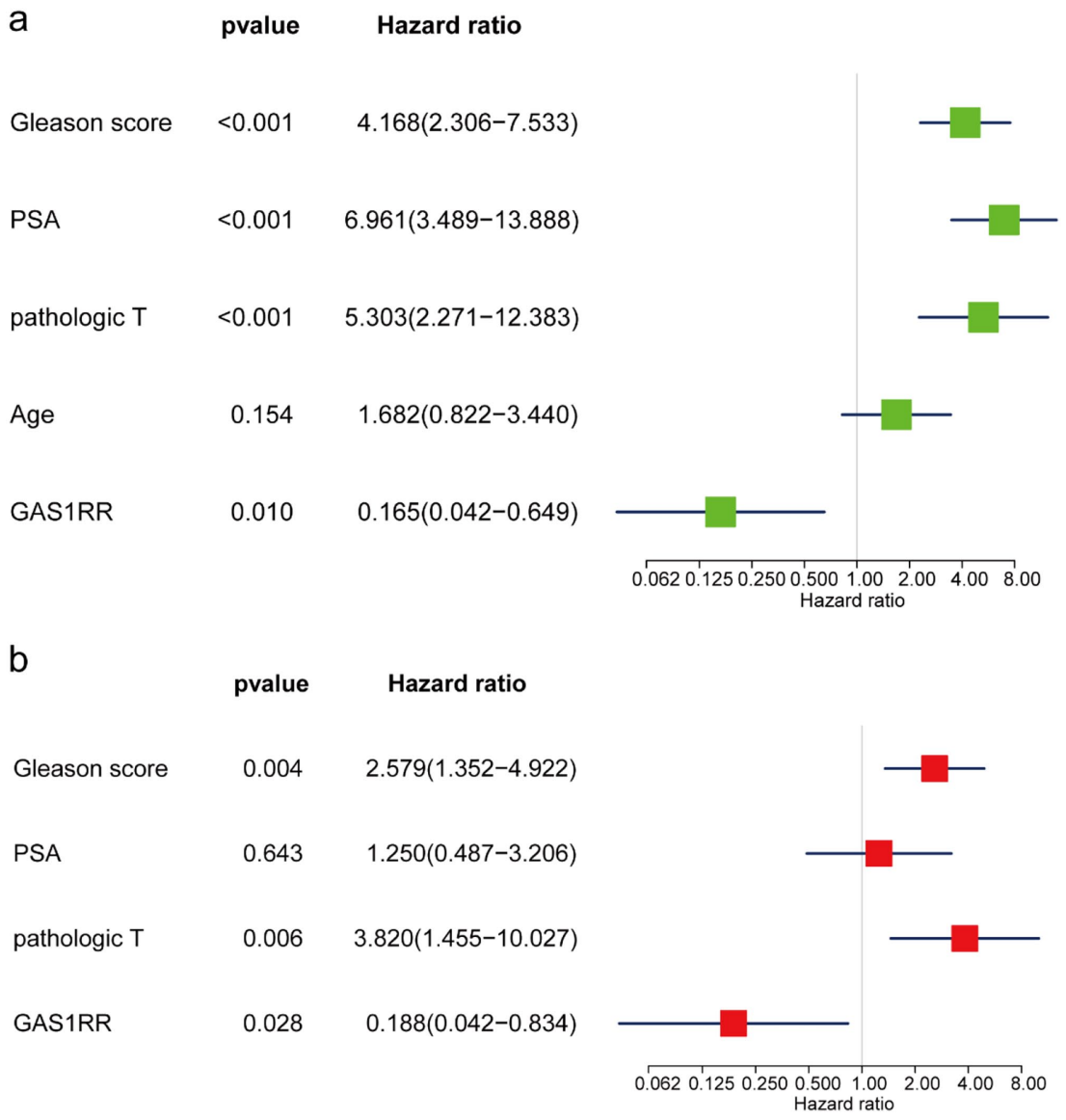

Figure 4. Forest plot of Cox regression analysis in PCa. (a) Univariate Cox regression analysis representing by forest plot and (b) multivariate Cox regression analysis representing by forest plot.

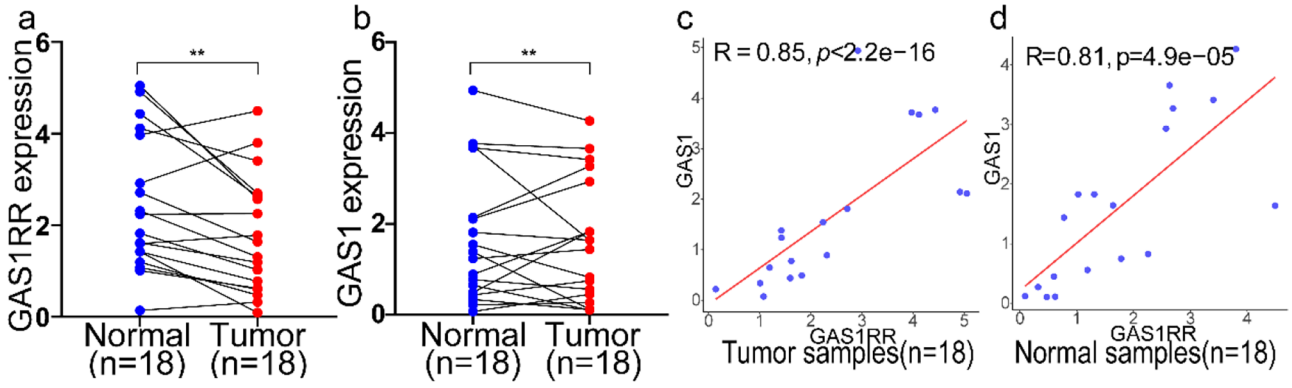

Figure 5. Verification of GAS1RR and GAS1 expressions in PCa tissues by RT-qPCR. (a) The level of GAS1RR expression is significantly lower in 18 pairs of prostate cancer tissues compared with normal tissues, (b) the level of GAS1 expression is significantly lower in 18 pairs of prostate cancer tissues compared with normal tissues, (c) Spearman's correlation analysis showed the strong positive correlation of GAS1RR and GAS1 in tumor prostate tissues, and (d) Spearman's correlation analysis showed the strong positive correlation of GAS1RR and GAS1 in normal prostate tissues.
$^{**}P < 0.001$.

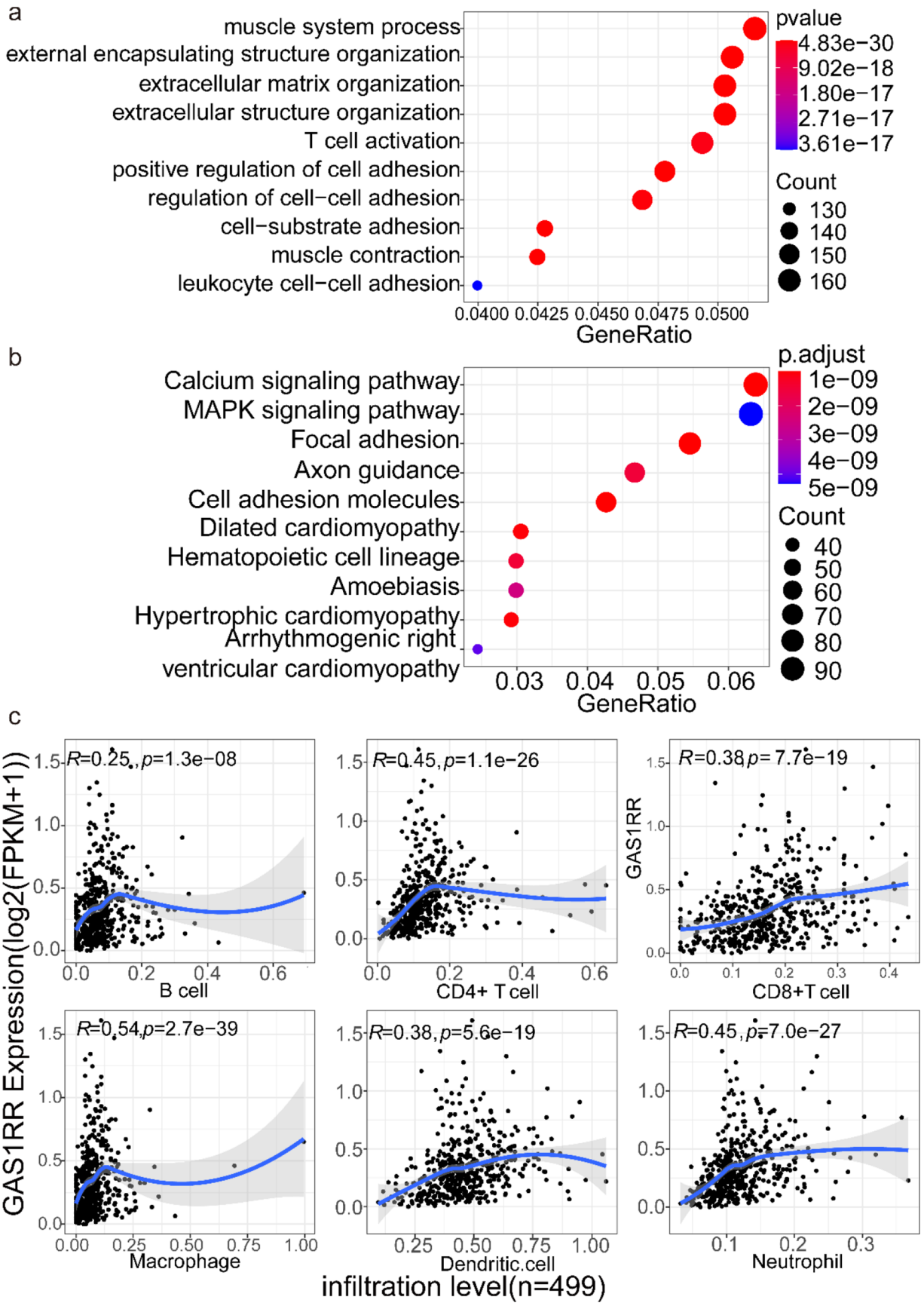

Figure 6. Biological functional enrichment analysis. (a) The GO analysis consequence of putative targets of GAS1RR displaying by bubble plot, (b) the KEGG analysis consequence of putative targets of GAS1RR displaying by bubble plot, and (c) the correlation analysis of immune infiltration levels of six kinds of important immune cells and GAS1RR expression by TIMER database.

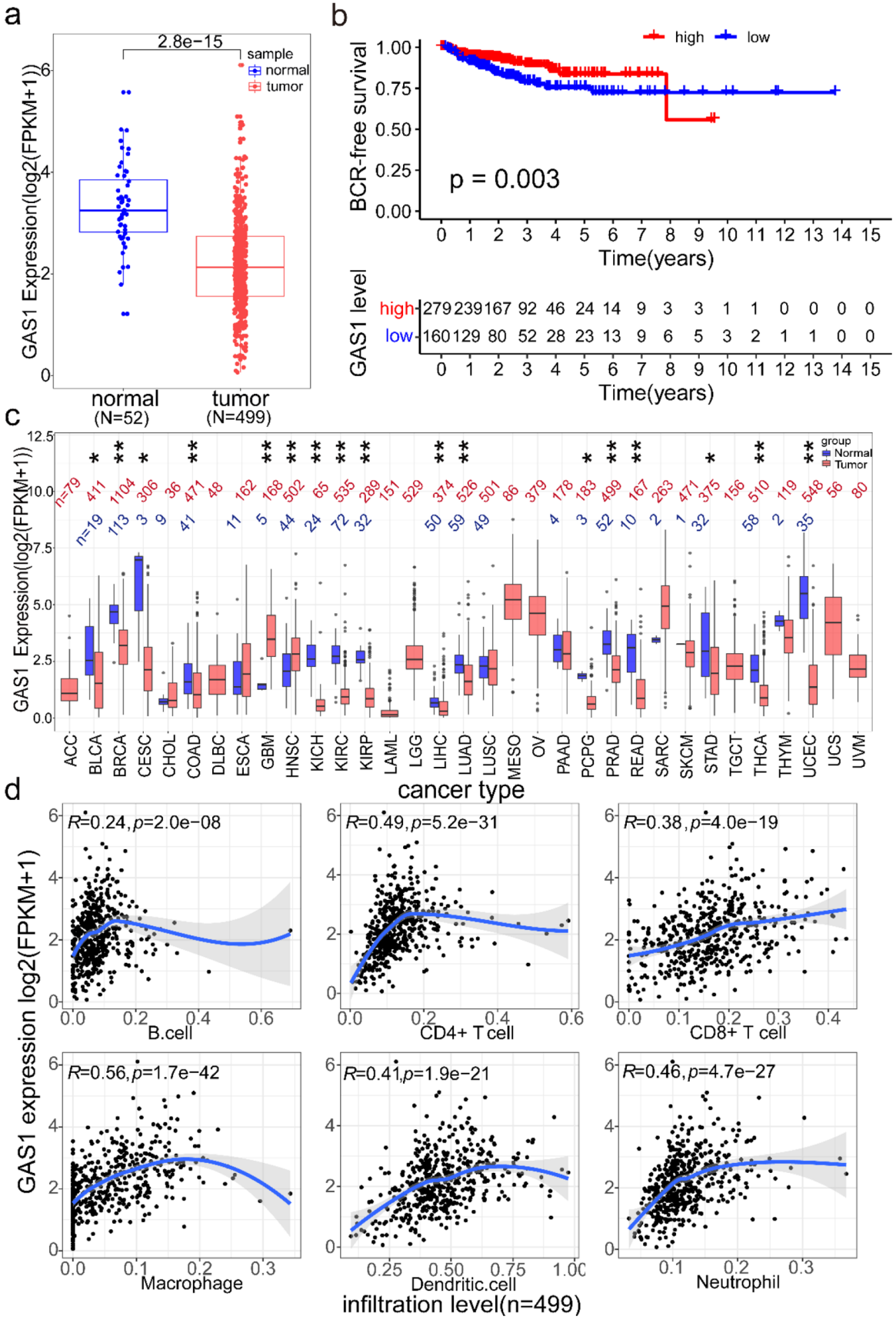

Figure 7. The correlation between GAS1 expression and immune infiltration. (a) GAS1 expression is significantly lower in PCa tissues when compared with normal ones, (b) Kaplan–Meier survival in high GAS1 and low GAS1 expression groups among TCGA cohorts, (c) expression profiles of GAS1 in 33 types of cancers from UCSC data set, and (d) the correlation analysis of immune infiltration levels of six kinds of important immune cells.

$P = 4.7e{-}27$) infiltration, which indicated that it may play an important role in immune regulation.

Discussion

In this article, we made an attempt to seek out eRNAs relevant with PCa as possible biomarkers for prognosis prediction and treatment determination of PCa. As far as we know, it is the first study indicating that GAS1RR, an eRNA, was disordered in PCa tissue. Besides, GAS1RR was positively correlated with GAS1, which may be related to the progress of PCa.

Although treatment options for PCa have greatly expanded over the past decade.[21] Current therapy options containing RP and RT are effective remedies for localized cancer.[3] Despite the fact that PCa patients possess a comparatively speaking better ending, recurrent PCa after primary surgery is likely to have disease progression and even metastasize distantly. Approximately 30% of patients had BCR. Without follow-up therapy, patients with biochemical relapse will have clinical progression within 5 years or so, and approximately 40% will die of PCa in less than 15 years.[22,23] Thus, it is of vital importance to identify a key biomarker for the prognosis and the choice of optimal curative therapy. On account of the fact that high-throughput sequencing technology and bioinformatics are booming in recent years, many novel biomarkers have been identified. Among them, a class of non-coding RNA called eRNAs synthesized from active enhancers are leading the charge in the revolution.[6,7,24–26] eRNAs are generally produced from enhancer region distinguished by ascending histone modifications H3K27ac and H3K4me1 and descending H3K4me3. It is already known that when the level of H3K27ac and H3K4me1 is increasing, more eRNAs could be transcribed from the enhancer of their target genes. What's more, there is increasing evidence that disorders of eRNAs are closely related to a variety of functional disorders even carcinogenesis, and eRNAs are a potential new therapeutic target.

In this article, we applied a comprehensive data analysis method to ascertain pivotal eRNAs of PCa. By use of the computational algorithm pipeline PreSTIGE, we got a list of eRNAs as well as their putative targets from a previous published paper.[18] For the reason that the role of eRNAs had been rarely studied in PCa, we used these 2695 pairs of molecules as candidates to seek for crucial eRNAs in PCa. Then, we performed Kaplan–Meier survival and correlation analysis, and found that GAS1RR was highly correlated with GAS1. The decline of GAS1RR expression was markedly interrelated with disadvantageous clinicopathological traits including higher PSA value, shorter time to BCR, higher Gleason score and higher pathologic T stage. The Kaplan–Meier survival analysis showed that downregulated GAS1RR expression was remarkably associated with shorter time to BCR. Furthermore, Cox regression analysis demonstrated that GAS1RR had an independent prognostic prediction value in PCa.

In cancer, GAS1 is a putative tumor suppressor which was first isolated from fibroblasts after serum withdraw and blocks cells entry to S phase, preventing the cycling of both normal and transformed cells. GAS1 expression is different during embryogenesis, and its expression is related to cell death during limb development.[14,15,27–31] GAS1RR is a regulatory long non-coding RNA which is adjacent to GAS1. Although very few reports are available to help us understand the function of GAS1RR, we infer that GAS1RR may also act as a cancer suppressor gene because it strongly co-expressed with GAS1. In addition, we discovered that the expression of GAS1RR declined significantly in tumor tissues compared to normal counterparts.

To verify the aforementioned results, we made use of data of 32 other types of cancer from TCGA as internal validation data sets and performed RT-qPCR of 18 pairs of PCa and normal specimens as external experimental validation. The consequence of pan-cancer analysis indicated that significant expression and survival differences of GAS1RR existed in many kinds of cancers. The results of RT-qPCR further certified the decline of GAS1RR in PCa. All the results were concordant, suggesting that GAS1RR may play a role in tumor suppression in PCa.

The results of GO and KEGG analyses have thrown some light on the means of GAS1RR influencing patients' BCR-free survival and prognosis. Results revealed that cell adhesion and structure organization were enriched, suggesting that GAS1RR alters the structure of the cancer cells. In the KEGG pathway analysis, calcium, MAPK signaling pathway, and focal adhesion were enriched. Furthermore, calcium signaling pathway and MAPK signaling pathway are both classical signaling pathways that have important functions in the process of carcinogenesis.[32–36] Alternatively, the change of cell structure is a novel marker of cancers.[37] Thus, we conjectured that GAS1RR may change cell structure through regulating many classical signal pathways so as to influence the development and prognosis of PCa.

Recently, more and more evidence indicates that tumor microenvironment is greatly affecting the progression of PCa. Immunotherapy, including tumor vaccination and immune checkpoint inhibitors (ICIs), has become a treatment option for cancer with great promise.[38–41] For instance, sipuleucel-T is the first commercial autologous cell-based vaccination immunotherapy, which was found to be effective against metastatic castration-resistant prostate cancer (mCRPC). In addition, ICIs are under investigation for mCRPC, and it has shown efficacy in the treatment of a variety of diseases including PCa, and ICIs may have an effect on mCRPC patients.[39,40] Furthermore, mounting proofs have emphasized the essential regulatory effects of eRNA in the immune system and eRNA-targeted therapy in cancer,[42–46] which indicates that it has a promising prospect in the therapeutic choices of PCa. For example, oncogene-induced eRNAs can promote tumorigenesis under certain circumstances, such as KLK3e in PCa, an androgen receptor (AR)–induced eRNA. KLK3e can regulate the expression of AR-dependent genes by scaffolding the AR-associated protein complex through regulating the expression of KLK3. Also, tumor suppressors can induce eRNAs to participate in the course of tumor suppression. For example, TP53-induced eRNAs could participate in p53-dependent stasis of cell cycle in many kinds of tumor cell lines.[46]

Immune infiltration analysis indicated that GAS1RR and GAS1 may also influence many kinds of immune cells to promote the carcinogenesis and progression of PCa. On account

of the fact that ICIs are correlated with T cell and the strong correlation between GAS1RR and CD4+ T cell, CD8+ T cell, we consider GAS1RR as an essential eRNA in immune regulation and may play a pivotal role in the regulation of microenvironment of PCa by functioning through its target gene *GAS1* and may have great potential to provide an additional avenue for the treatment of PCa patients.

In spite of the fact that this study figured out the clinical value of GAS1RR in PCa, there still remains several limitations to be taken into account. First, our public acquired data sets were mainly from TCGA, and the majority of people are white race, so there is likely to be racial bias. Second, the number of patient samples enrolled in the paper is small, so the representativeness maybe not satisfying. Third, the regulatory mechanisms between GAS1RR and GAS1 should be clarified.

Conclusions

To sum up, this study screened out a vital eRNA GAS1RR related with BCR-free survival in PCa patients, which plays a pivotal role in PCa. It turned out that lower GAS1RR expression implied a poorer prognosis for PCa patients. GAS1 is a putative target of GAS1RR, and GAS1RR may function through enhancing GAS1 expression. Our research provides a new insight to look at the pathogenesis of PCa, and GAS1RR possesses the potential to become a prospective forecasting factor for PCa, which may benefit PCa patients in the future.

AUTHORS' CONTRIBUTIONS

ZZX designed the study, performed experiments, and finish the manuscript. YG revised the manuscript. SM and JX analyzed the data. LL and YNW generated the tables and pictures. LNL contributed to the editing of the manuscript. BL, CGY, YL, BLQ, and ZQY revised the manuscript. All the authors participated in the design interpretation of the article and accepted the final version of the article.

DECLARATION OF CONFLICTING INTERESTS

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DATA AVAILABILITY STATEMENT

All the pan-cancer expression and relative survival data can be downloaded from UCSC Xena database (https://xenabrowser.net/datapages/), and a list of putative eRNAs and their targets were acquired from a publish paper (Vučićević *et al*.[18]). The raw data of RT-qPCR can be acquired from the Supplementary Files.

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SUPPLEMENTAL MATERIAL

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