Am J Cancer Res 2022;12(4):1824-1842
www.ajcr.us /ISSN:2156-6976/ajcr0142330

Original Article

Oncogenic ACSM1 in prostate cancer is through metabolic and extracellular matrix-receptor interaction signaling pathways

Yongchen Guo[1], Chunna Ren[2], Wentao Huang[3], Wancai Yang[4], Yonghua Bao[5]

[1]Department of Immunology, Mudanjiang Medical University, Mudanjiang 157011, China; [2]The Second Affiliated Hospital of Mudanjiang Medical University, Mudanjiang 157011, China; [3]Hongqi Hospital Affiliated to Mudanjiang Medical University, Mudanjiang 157011, China; [4]Department of Pathology, University of Illinois at Chicago, IL 60612, USA; [5]Department of Pathology, Mudanjiang Medical University, Mudanjiang 157011, China

Received February 13, 2022; Accepted April 1, 2022; Epub April 15, 2022; Published April 30, 2022

Abstract: Acyl-coenzyme A synthetase medium chain family member 1 (ACSM1) is a medium chain Acyl-CoA Synthetase family member and plays an important role in fatty acid metabolism. The oncogenic roles of ACSM1 are largely unknown. Using comprehensive approaches, we analyzed gene expression profiles and genomic datasets and identified that the expression of ACSM1 was specifically increased in prostate cancer in comparison to the adjacent non-tumor tissues. The increased expression of ACSM1 was associated with increased risks of poor prognosis and shorter survival time. Moreover, genomic copy number alterations of ACSM1, including deletion, amplification, and amino acid changes were frequently observed in prostate cancers, although these mutations did not correlate with gene expression levels. However, ACSM1 gene amplifications were significantly corrected with increased risks of prostate cancer metastasis, and ACSM1 genetic alterations were significantly associated with worse disease-free. And progress-free survival. Gene function stratification and gene set enrichment analysis revealed that the oncogenic roles of ACSM1 in prostate cancer were mainly through metabolic pathways and extracellular matrix (ECM)-receptor interaction signaling pathways, but not associated with microenvironmental immunological signaling pathways, and that ACSM1 expression was not associated with immune cell infiltration in the cancer microenvironment or prostate cancer immune subtypes. In conclusion, the present work has demonstrated that ACSM1 can be specifically and significantly elevated in prostate cancer. ACSM1 gene expression and genomic amplification exhibit important clinical significance through metabolic and ECM-receptor interaction signaling pathways. Thus, ACSM1 may be a novel oncogene and serve as a biomarker for prostate cancer screening and prognosis prediction, and/or a therapeutic target.

Keywords: ACSM1, prostate cancer, metastasis, microenvironment, metabolic signaling pathway, ECM-receptor interaction

Introduction

ACSM1 (Acyl-coenzyme A synthetase medium chain family member 1) is a medium chain Acyl-CoA Synthetase family member (also known as MACS1). Located in 16p.12.3, ACSM1 encodes a protein of 577 amino acids with an estimated molecular weight of 65 kDa [1]. This enzyme catalyzes the activation of fatty acids by CoA to produce acyl-CoA, the first step in fatty acid metabolism [2-4] and plays an important role in the metabolic system [5, 6]. Its clinical significance has not been well characterized. Some reports have suggested that ACSM1 is a genetic predisposition gene for major depressive disorder (MDD) and Schizophrenia (SCZ), two of the most common and severe mental disorders [7]. It has also been reported that differential expression of ACSM1 is observed in breast apocrine carcinomas [8]. However, ACSM1 expression levels and biological functions in cancer are largely unknown.

Recent studies from the combination of gene profiling, proteomics and metabolomics, genomic technologies, and bioinformatic analysis

have identified numerous genes whose expression is up- or down-regulated in cancers [3, 9-12]. However, the specificity and sensitivity for prostate cancer are not clear. We have recently re-analyzed the prostate cancer gene profile data from The Cancer Genome Atlas (TCGA) and from other studies on prostate cancer and found differential gene expression and genomic alterations. However, the expression levels of very few genes, either upregulated or downregulated, are specific in prostate cancer. ACSM1 was among those genes whose expression level is significantly increased in prostate cancer tissues, compared to the adjacent non-cancerous or normal tissues.

Prostate cancer is the most common cancer and third leading cause of cancer death in men worldwide [13]. It is estimated that in 2022, both incidence and mortality rates are slightly increased from last year [13, 14]. And there will be approximately 268,490 new cases of prostate cancer in the United States alone, accompanied by an estimated 34,500 deaths from prostate cancer [13], mainly due to a lack of efficient early detection biomarkers and efficient targets for personalized or precision therapy. Although the prostate specific antigen (PSA) is a well-known and widely used biomarker for prostate cancer, its clinical utilization has also caused concerns of inconsistency with clinic-pathological characteristics [15-17]. For instance, the levels of PSA are not consistent with Gleason scores, and do not show prognostic significance in tumor metastasis [18, 19]. It has been noted that prostate cancer-associated death is not from the cancer itself, but from cancer metastasis and the dysfunction of affected organs [20-22]. Therefore, the identification of novel biomarkers for prostate cancer diagnosis and prediction of progression, recurrence, and metastasis is sorely needed. Moreover, revealing the underlying mechanisms will improve the understanding of prostate cancer development and progression, providing alternative therapeutic strategies for precision oncology.

The present study also showed that increased expression of ACSM1 was correlated with poor outcomes, but not with cancer environment immune cell infiltration, prostate cancer immune subtypes and molecular classifications. Furthermore, genomic copy number alterations (CNAs) were frequently seen in prostate cancers, but the amplifications were associated with increased risks of cancer metastasis. Moreover, gene function stratification and gene set enrichment analysis revealed that the underlying mechanisms of oncogenic roles of ACSM1 in prostate cancer to be mainly through metabolic pathways and extracellular matrix (ECM)-receptor interaction signaling pathways.

Materials and methods

TCGA and ONCOMINE data information and survival analysis

The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/) and ONCOMINE™ Platform (https://www.oncomine.org) are open-source databases for researchers. We used the GDC-CLIENT software to download 551 prostate cancer (PRAD) FPKM data files from TCGA website (Version 07-20-2019, including 485 adenomas and adenocarcinoma cases, 9 ductal and lobular neoplasm cases, 1 cystic, mucinous and serous neoplasm case). Among these, 495 cases have vital status (483 alive, 10 dead, 2 not reported). For the ONCOMINE website, prostate cancer expression profile datasets from multiple studies were accessed. The ACSM1 gene expression level and corresponding clinical information, including primary and metastatic cancer status, were retrieved and analyzed.

For survival analysis, The Human Protein Atlas data set (https://www.proteinatlas.org/) was used. A total of 494 prostate cancer cases from TCGA dataset were used, including 377 cases with low ACSM1 expression and 117 cases with high ACSM1 expression.

Gene expression analysis

TCGA differential gene expression analysis was processed using multiple online databases. Differential gene expression was analyzed on the GEPIA (Gene Expression Profiling Interactive Analysis) website http://gepia.cancer-pku.cn/index.html, an interactive web server for analyzing RNA sequencing data from TCGA and GTEx projects, providing web-based analytical functions of clinical data using a standard processing pipeline [23]. Gene expression levels were defined with [Log_2FC] >1 and value *<0.01* as the cut-off in different cancers. R lan-

guage was applied in medical statistical analysis. R packages (Version 3.6.3, https://cran.r-project.org/web/packages/) (including the ggplot2 package) was used to calculate prorate cancer (PRAD) paired samples and unpaired samples for differential expression analysis with the Wilcoxon rank sum test and distinctive mark (P≥0.05; *, P<0.05; **, P<0.01; ***, P<0.001). The Shapiro-Wilk normality test and Spearman statistical methods were used in molecular correlation analysis. Receiver operating characteristic curve (ROC) was processed with PROC package (Version 1.17.0.1) and ggplot2 package, where the abscissa is the false positive rate (FPR) and the ordinate the true positive rate (TPR). Gene baseline characteristics of TCGA prostate cancer were analyzed depending on theoretical frequency, sample size and normal distribution with Chi-square test in T stage and N stage, Fisher test in M stage, and Wilcoxon rank sum test in Age.

ACSM1 gene mutation signature research and genomic alteration analysis

For ACSM1 gene mutation signature research and genomic alteration analysis, prostate cancer datasets were selected from the cBioPortal database (www.cbioportal.org) [24]. Genomic information was retrieved and a total of 7,161 cases of prostate cancer were analyzed, where 6,082 and 1,079 cases were without and with metastasis respectively. Genomic alterations, including DNA copy number variants (CNVs), genomic amplifications and deletions, and amino acid changes, were calculated. The odds ratio (OR) and 95% CI (confidence interval) were statistically analyzed by comparing prostate cancer groups with vs. without metastasis. *P* values of less than 0.05 was considered significant. Relationship data between mutation frequency, type, and site are obtained through different modules. The copy number, mutation status, and patient survival of various cancer types are also analyzed using datasets from multiple studies retrieved from cBioPortal.

Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an *a priori* defined set of genes shows statistically significant, concordant differences between two biological states [25, 26]. GSEA analysis was carried out through the WebGestalt online website (WEB-based GEne SeT AnaLysis Toolkit) (http://www.webgestalt.org/), a functional enrichment analysis web tool that supports GSEA [25, 27]. Prostate cancer (PRAD) differentially expressed genes (DEGs) data were analyzed on the WebGestalt website with website-advanced statistical parameters, and the results were further evaluated by the clusterProfiler R package.

Gene Ontology (GO)/KEGG analysis

The Gene Ontology (GO) analysis can describe aspects of a gene's biology. The clusterProfiler R package is an ontology-based tool that offers different methods for gene classification and enrichment analyses [28]. GO analysis and significantly enriched GO terms were implemented by the clusterProfiler R package for differentially expressed genes data in TCGA prostate cancer, with cutoffs of *p*-values <0.01 and FDR <0.05. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted using the clusterProfiler R package.

Protein-protein interaction (PPI) network complex analysis

The online database STRING (http://string-db.org) [29] was used to query ACSM1-associated genes and protein-protein interaction networks (PPI) with the criterion. The Cytoscape software [30] was utilized to construct protein interaction and relationship network with STRING output results, and the modules of ACSM1-associated genes were filtered with the MCODE (Molecular Complex Detection) plugin in Cytoscape 3.7.2 (Standard default: Degree cutoff =2, node score cutoff =0.2, k-core =2, and max depth =100).

Gene expression and abundance of immune infiltrates and subtype analysis

The TIMER web server is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (https://cistrome.shinyapps.io/timer/) [31]. ACSM1 gene Tumor Immune Estimation was evaluated with B cells, CD4+ T cells, CD8+ T cells, Neutrophils, Macrophages, and Dendritic cells. Subtype distribution was submitted to the TISIDB website in PRAD (http://cis.hku.hk/TISIDB/) [32].

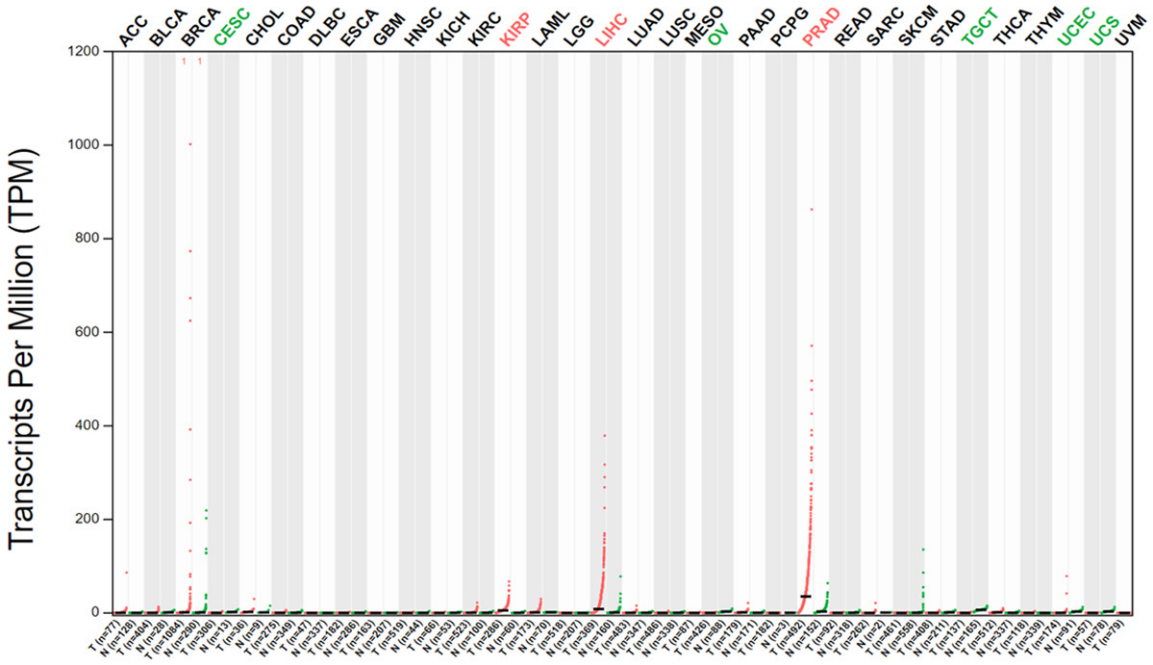


Figure 1. ACSM1 gene expression profile in 33 types of cancers. The words in red stood for upregulated expression, the words in green stood for downregulated expression, and the words in black stood for non-changed expression. Cancer types abbreviations: ACC, Adrenocortical carcinoma; BLCA, Bladder urothelial carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangial carcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid neoplasm diffuse Large B-cell lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and neck squamous cell carcinoma; KICH, Kidney chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute myeloid leukemia; LGG, Brain lower grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin cutaneous melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular germ cell tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine corpus endometrial carcinoma; UCS, Uterine carcinosarcoma; UVM, Uveal melanoma.

The association between ACSM1 expression and prostate cancer molecular subtypes was evaluated using the TCGA prostate cancer dataset based on classifications derived from the literature [33-35] which are ETS family transcription factors (ERG, ETV1, ETV4, and FLI1), non-ETS factors (SPOP, FOXA1, and IDH1), and others (PI3K, p53, CHD1, and SPINK1).

Results

ACSM1 was differentially expressed in pan-cancers and prostate cancer exhibited the highest expression level of ACSM1

The Cancer Genome Atlas (TCGA) has provided tremendous information at genomic, genetic, transcriptional, and translational levels. Through deep data mining of the TCGA gene expression profile data across all tumors and non-tumor tissues, we found that ACSM1 mRNA levels were differentially expressed in 33 different cancer types (**Figure 1**). However, pan-cancer profiles showed that among cancers with upregulated expression, prostate cancer exhibited the highest expression levels of ACSM1, suggesting the specificity of ACSM1 in prostate cancer.

ACSM1 was significantly increased in prostate carcinomas and metastatic prostate cancer

To detail the expression of ACSM1 in prostate cancer, we re-analyzed the prostate cancer datasets from TCGA and other studies. As shown in **Figure 2A**, ACSM1 was significantly increased in prostate cancer in comparison to non-tumor tissues. Among the 499 cases of prostate cancer in the TCGA dataset, 52 cases had both cancer and matched normal tissues. Again, prostate cancer tissues showed signifi-

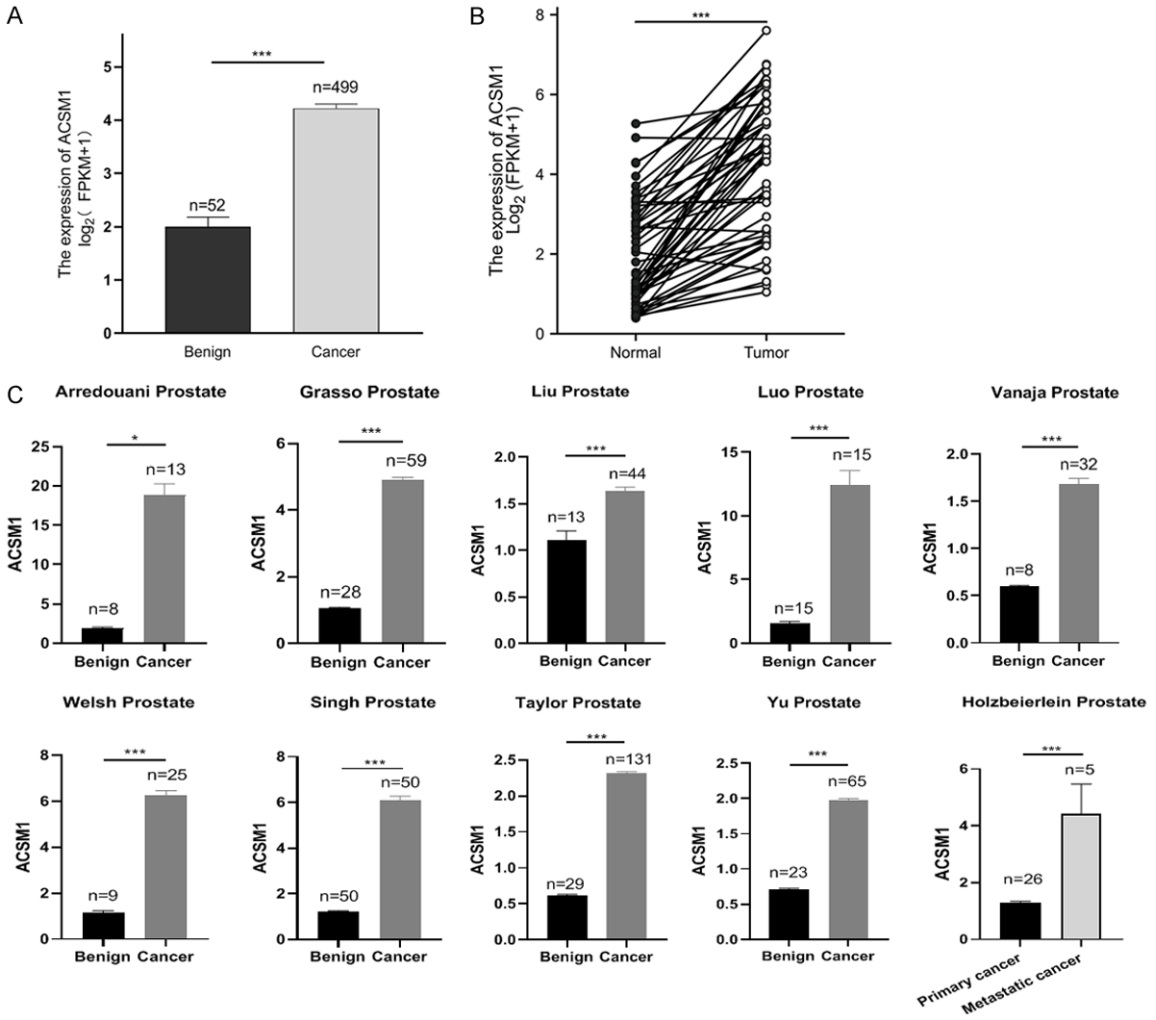


Figure 2. The expression levels of ACSM1 were significantly upregulated in prostate cancers in comparison to non-cancer tissues and increased in metastatic prostate cancers in comparison to the primary cancers. A. Expression levels of ACSM1 in TCGA prostate cancers (Normal or benign cases =52, cancer cases =499; ***P<0.001). B. Expression levels of ACSM1 in prostate cancers and matched non-tumor tissues from TCGA datasets (Normal: cancer =52: 52; ***P<0.001). C. ACSM1 expression in prostate cancers from other nine studies from Oncomine datasets (*P<0.05; ***P<0.001); And ACSM1 expression level was increased in metastatic prostate cancers, in comparison to the primary cancers (***P<0.001).

cant increases of ACSM1 expression in the paired samples (**Figure 2B**).

Data analysis from another nine studies [36-41] from the Oncomine database also showed significant increases of ACSM1 expression in prostate cancer, compared to benign or normal prostate tissues (**Figure 2C**).

To elucidate the association between gene expression and metastasis, one set of metastatic prostate cancer gene expression data from the Holzbeierlein study was analyzed [42]. In comparison to the primary cancer, metastatic prostate cancer exhibited higher expression of ACSM1 (**Figure 2C**).

Genomic alterations of ACSM1 increase the risk of prostate cancer metastasis

Clinical studies have shown important roles of genetic alterations in prostate cancer metastasis. We utilized the web-based datasets (www.cbioprtal.org) and conducted a comprehensive analysis in a total of 7,161 cases of prostate cancer and 1,079 cases that had metastasis. As shown in **Table 1**, we found that genomic alterations of ACSM1 significantly increased the risk of metastasis, with an odds ratio (OR) of 6.22 (95% CI: 3.60, 10.76), which was mainly due to copy number variants (CNVs). For genomic amplifications, the odds ratio increased to

Table 1. ACSM1 copy number variations and their association with prostate cancer metastasis

Total (%)-mutation types			Prostate cancer without metastasis			Prostate cancer with metastasis			Odds ratio (95% confidence interval)	*p* value*
	Altered numbers (%)	Non-altered numbers (%)	Sub-total (%)-mutation types	Altered numbers (%)	Non-altered numbers (%)	Sub-total (%)-mutation types	Altered numbers (%)	Non-altered numbers (%)		
7161 (100)	52 (0.7)	7109 (99.3)	6082 (100)	25 (0.4)	6057 (99.6)	1079 (100)	27 (2.5)	1052 (97.5)	6.22 (3.60, 10.76)	<0.0001
Copy number variants (CNVs)			Copy number variants (CNVs)			Copy number variants (CNVs)				
Amplification	43 (82.7%)		Amplification	19 (76.0%)		Amplification	24 (88.9%)		7.26 (3.96, 13.30)	<0.0001
Deletion	5 (9.6%)		Deletion	2 (8.0%)		Deletion	3 (11.1%)			NS
Protein changes			Protein changes			Protein changes				
D57E (Missense)	1 (1.9%)		D57E (Missense)	1 (4.0%)						
P233H (Missense)	1 (1.9%)		P233H (Missense)	1 (4.0%)						
V347M (Missense)	1 (1.9%)		V347M (Missense)	1 (4.0%)						
H240Y (Missense)	1 (1.9%)		H240Y (Missense)	1 (4.0%)						
Sub-total (%)	52 (100%)		Sub-total	25 (100%)			27 (100%)			

*Comparison between without metastasis and with metastasis groups. NS, no significance.

7.26 (95% CI: 3.96, 13.30). This finding was supported by other studies showing that metastatic prostate cancer exhibited the highest percentages of ACSM1 genomic amplifications (**Figure 3A**).

In addition, genomic alterations of ACSM1 were also analyzed on TCGA Pan-Cancer Atlas studies from 10,953 patients/10,967 samples in 35 studies. As shown in **Figure 3B**, the mutations were frequently observed in multiple cancer types. Besides prostate cancer, cancer types including breast cancer, bladder cancer, and diffuse large B-cell lymphoma also showed gene amplifications as the dominant alterations. Mutation sites were distributed throughout the gene but concentrated in the AMP-binding domain (**Figure 3C**). Unexpectedly, these alterations did not affect gene expression, as evidenced by the lack of significant association between ACSM1 gene expression and copy number alterations, compared to diploid samples in 8,521 prostate cancer samples from 22 studies (**Figure 3D**).

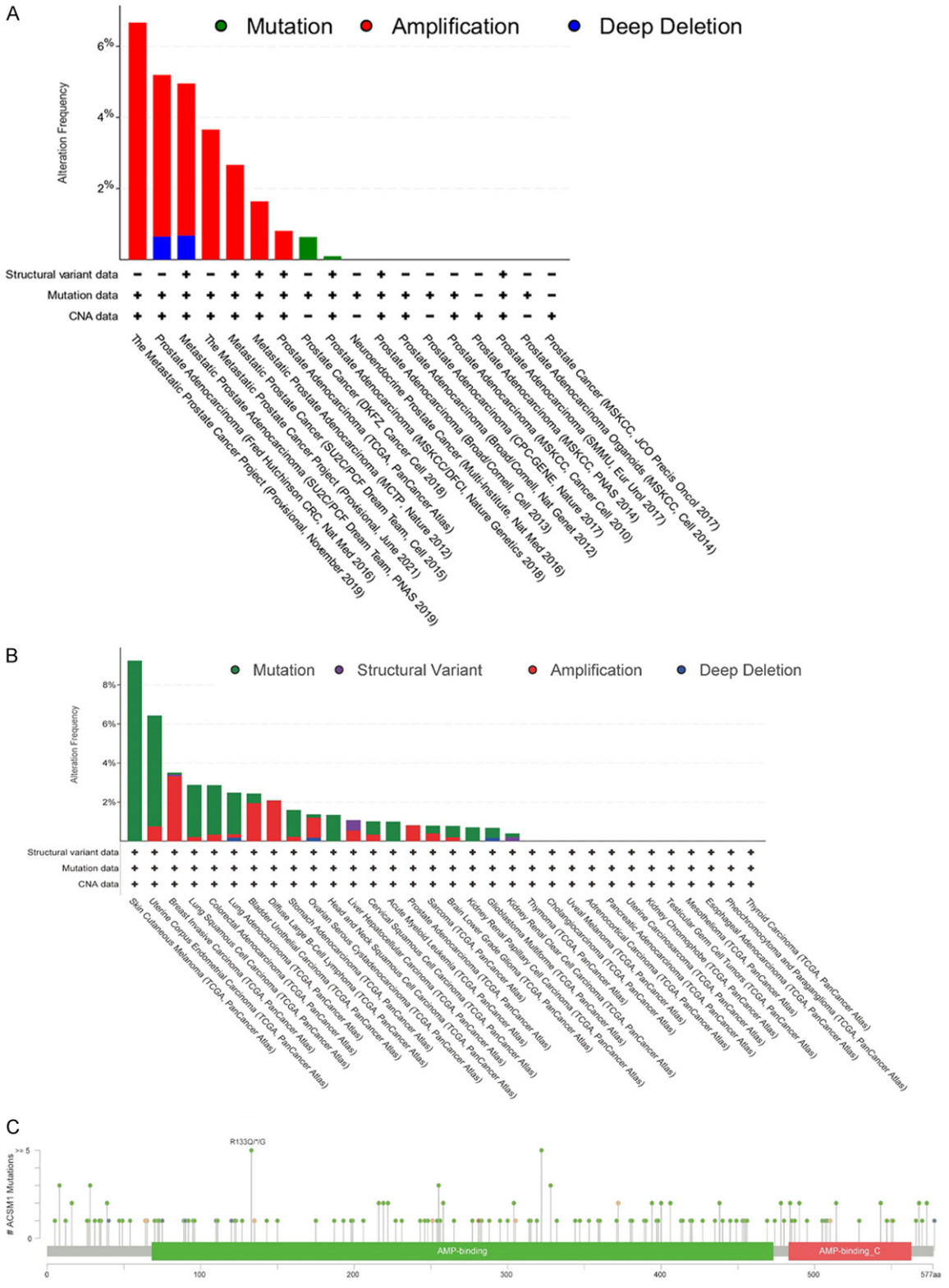
Prognostic significance of ACSM1 in prostate cancer

The increased expression in prostate cancer and the increase of metastasis by genomic alterations led us to determine the prognostic significance of ACSM1. As shown in **Figure 4A**, ACSM1 expression level increased the risk of prostate cancer progression, with the area under curve (AUC) =0.828 and confidential interval (CI) =0.778-0.878, and high expression of ACSM1 showed a trend of shorter survival time (**Figure 4B**, P>0.05). Moreover, the correlation between genetic alterations of the ACSM1 gene and survival was also analyzed using the big dataset from cBioPortal (www.cbioportal.org). We found that ACSM1 genetic alterations were significantly associated with disease-free survival ($P=9.74\times10^{-3}$) and progress-free survival ($P=8.62\times10^{-3}$), but not with overall survival (P>0.07) (**Figure 4C**). The association between ACSM1 expression and prognosis in pan-cancers has not been well characterized and needs further investigation.

ACSM1-associated oncogenic roles were through metabolic and ECM-receptor interaction signaling pathways

To determine the underlying mechanisms of ACSM1-associated oncogenic roles in prostate cancer, we conducted gene functions and gene set enrichment analysis (GSEA). Several cancer-related pathways were enriched and significantly associated with ACSM1 expression in prostate cancer. One group of signaling pathways was positively enriched with the expression of ACSM1 in prostate cancer, including Ribosome, Proteasome, N Glycan Biosynthesis, Peroxisome, Citrate TCA Cycle, Systemic Lupus Erythematosus, Valine Leucine and Isoleucine Degradation, Amino sugar and Nucleotide Sugar Metabolism, Protein Export, and Butanoate metabolism signaling pathways. The top 5 upregulated gene sets are shown in **Figure 5A**. In contrast, one group of signaling pathways was negatively enriched with the expression of ACSM1 in prostate cancer, including ECM-Receptor Interaction, Dilated Cardiomyopathy, Hypertrophic Cardiomyopathy HCM, Focal Adhesion, Arrhythmogenic Right Ventricular Cardiomyopathy Arvc, Aldosterone Regulated Sodium Reabsorption, Phosphatidylinositol Signaling System, Vascular Smooth Muscle Contraction, Small Cell Lung cancer, and Metabolism of Xenobiotics by Cytochrome P450 signaling pathways. The top 5 downregulated gene sets are shown in **Figure 5B**.

Gene functional stratification showed significantly enriched GO terms of differentially expressed genes (DEGs) from prostate cancer in TCGA database as shown in **Figure 5C**. GO analysis classified the DEGs into 3 groups, i.e. Molecular Function, Biological Process, and Cellular Component. In the Molecular Function group, the significantly changed and highest number of genes changed were extracellular matrix (ECM)-associated gene sets, including extracellular matrix structural constituent (29 of the total 596 genes, p value $=2.051\times10^{-13}$), and extracellular matrix structural constituent conferring tensile strength (14 of the total 596 genes, p value $=5.489\times10^{-11}$) (**Figure 5C**, left panel). In the Biological Process group, extracellular matrix organization (39 of the total 608 genes, p value $=1.7037\times10^{-11}$) and extracellular structure organization (43 of the total 608 genes, p value $=8.000\times10^{-12}$) were two of the most changed gene sets (**Figure 5C**, middle panel). Similarly, in the Cellular Component group, extracellular matrix (63 of the total 629 genes, p value $=3.212\times10^{-21}$) and collagen-containing extracellular matrix (58 of the total 629 genes, p value $=2.96\times10^{-21}$) were two of the


A
Mutation
Amplification
Deep Deletion
Alteration Frequency
6%
4%
2%
Structural variant data
Mutation data
CNA data
The Metastatic Prostate Cancer Project (Provisional, November 2019)
Prostate Adenocarcinoma (Fred Hutchinson CRC, Nat Med 2016)
Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team, PNAS 2019)
Metastatic Prostate Cancer Project (Provisional, June 2021)
The Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team, Cell 2015)
Metastatic Prostate Adenocarcinoma (MCTP, Nature 2012)
Prostate Adenocarcinoma (TCGA, PanCancer Atlas)
Prostate Cancer (DKFZ, Cancer Cell 2018)
Neuroendocrine Prostate Cancer (Multi-Institute, Nat Med 2016)
Prostate Adenocarcinoma (MSKCC/DFCI, Nature Genetics 2018)
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Prostate Adenocarcinoma (CPCGENE, Nature 2017)
Prostate Adenocarcinoma (Broad/Cornell, Nat Genet 2012)
Prostate Adenocarcinoma (MSKCC, Cancer Cell 2010)
Prostate Adenocarcinoma (MSKCC, PNAS 2014)
Prostate Adenocarcinoma Organoids (SMMU, Eur Urol 2017)
Prostate Cancer (MSKCC, JCO Precis Oncol 2017)
Prostate Adenocarcinoma (MSKCC, Cell 2014)
B
Mutation
Structural Variant
Amplification
Deep Deletion
Alteration Frequency
8%
6%
4%
2%
Structural variant data
Mutation data
CNA data
Skin Cutaneous Melanoma (TCGA, PanCancer Atlas)
Uterine Corpus Endometrial Carcinoma (TCGA, PanCancer Atlas)
Breast Invasive Carcinoma (TCGA, PanCancer Atlas)
Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas)
Colorectal Adenocarcinoma (TCGA, PanCancer Atlas)
Lung Adenocarcinoma (TCGA, PanCancer Atlas)
Bladder Urothelial Carcinoma (TCGA, PanCancer Atlas)
Diffuse Large B-Cell Lymphoma (TCGA, PanCancer Atlas)
Stomach Adenocarcinoma (TCGA, PanCancer Atlas)
Ovarian Serous Cystadenocarcinoma (TCGA, PanCancer Atlas)
Head and Neck Squamous Cell Carcinoma (TCGA, PanCancer Atlas)
Liver Hepatocellular Carcinoma (TCGA, PanCancer Atlas)
Cervical Squamous Cell Carcinoma (TCGA, PanCancer Atlas)
Acute Myeloid Leukemia (TCGA, PanCancer Atlas)
Prostate Adenocarcinoma (TCGA, PanCancer Atlas)
Sarcoma (TCGA, PanCancer Atlas)
Brain Lower Grade Glioma (TCGA, PanCancer Atlas)
Kidney Renal Papillary Cell Carcinoma (TCGA, PanCancer Atlas)
Glioblastoma Multiforme (TCGA, PanCancer Atlas)
Kidney Renal Clear Cell Carcinoma (TCGA, PanCancer Atlas)
Thymoma (TCGA, PanCancer Atlas)
Cholangiocarcinoma (TCGA, PanCancer Atlas)
Uveal Melanoma (TCGA, PanCancer Atlas)
Adrenocortical Carcinoma (TCGA, PanCancer Atlas)
Pancreatic Adenocarcinoma (TCGA, PanCancer Atlas)
Uterine Carcinosarcoma (TCGA, PanCancer Atlas)
Kidney Chromophobe (TCGA, PanCancer Atlas)
Testicular Germ Cell Tumors (TCGA, PanCancer Atlas)
Mesothelioma (TCGA, PanCancer Atlas)
Esophageal Adenocarcinoma (TCGA, PanCancer Atlas)
Pheochromocytoma and Paraganglioma (TCGA, PanCancer Atlas)
Thyroid Carcinoma (TCGA, PanCancer Atlas)
C
R133Q/*/G
ACSM1 Mutations
>= 5
0
AMP-binding
AMP-binding_C
0
100
200
300
400
500
577aa

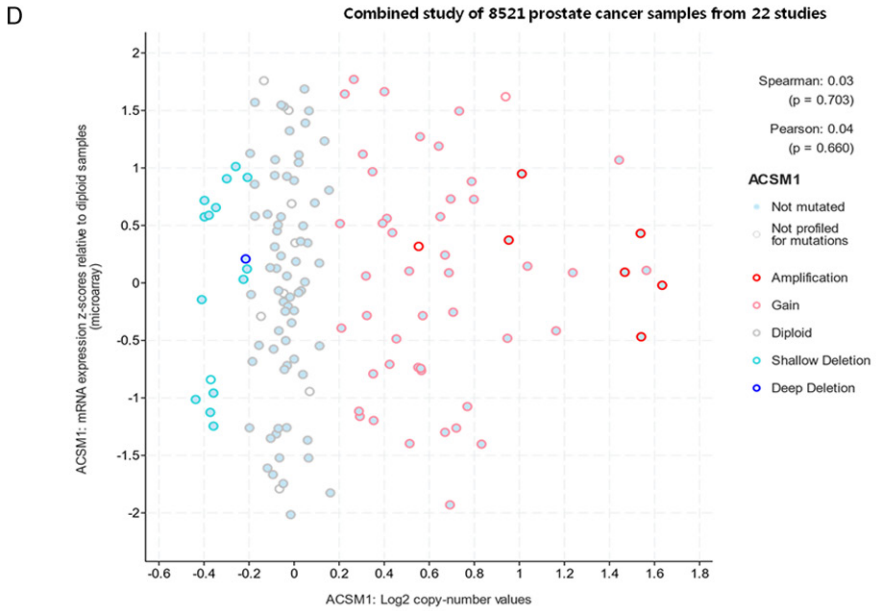


Figure 3. Genomic alterations of ACSM1 increase the risk of prostate cancer metastasis. A. Genomic alterations in prostate cancers. Metastatic prostate cancers exhibited the highest percentage of ACSM1 amplifications. B and C. Mutation frequency, mutation type and mutation site analysis in 33 of 35 categories (Pan-cancers) from cBioPortal database. D. ACSM1 copy number changes were not associated with mRNA expression compared with diploid samples, analyzed from a combined study of 8521 prostate cancer samples from 22 studies from cBioPortal database.

most changed gene sets (**Figure 5C**, right panel).

The Top 20 pathways from the KEGG database, including purine metabolism, thiamine metabolism, 2-Oxocarboxylic acid metabolism, biosynthesis of amino acids, and others, were also identified (**Figure 5D**). This again supported the association between ACSM1-involved metabolism and prostate cancer.

A search was conducted using the protein name ACSM1 and organism *Homo sapiens* in the STRING online database (http://string-db.org). A total of 47 ACSM1-interacting proteins that had been confirmed experimentally were then collected. Further analysis using Cytotype MCODE showed ACSM1-interacting proteins to be mainly associated with metabolic signaling pathways (**Figure 5E**).

ACSM1 expression was not associated with stromal immune cell infiltration, or with immune and molecular subtypes of prostate cancer

Since the cancer microenvironment and immune system play important roles in tumorigenesis, metastasis, and responses to therapy, we determined the roles of ACSM1 in prostate cancer stromal immune system. First, we explored the association between ACSM1 expression and immune cell infiltration in prostate cancer environment, but found no association between the expression levels of ACSM1 and the infiltration of B cells, CD8+ T cells, macrophage cells, neutrophil cells, or dendritic cells, except for a negative association with CD4+ T cell infiltration (Data not shown). Next, we determined the association between ACSM1 expression with immune subtypes of prostate cancer. Prostate cancer subtype distribution was submitted to the TISIDB website, in which prostate cancer can be divided into 4 immune subtypes (C1, C2, C3, and C4). However, we did not see significant association of ACSM1 expression levels with any of the subtypes of prostate cancer (Data not shown).

Increasing clinical studies have agreed that prostate cancer can be classified into 7 major molecular subtypes, including 4 gene fusions [ETS-related gene (TMPRSS2-ERG), ETS variant 1 (ETV1), ETV4, and FLI1] and 3 gene mutations (SPOP, FOXA1, and IDH1). Others include alterations in PI3K and p53 signaling, deletions

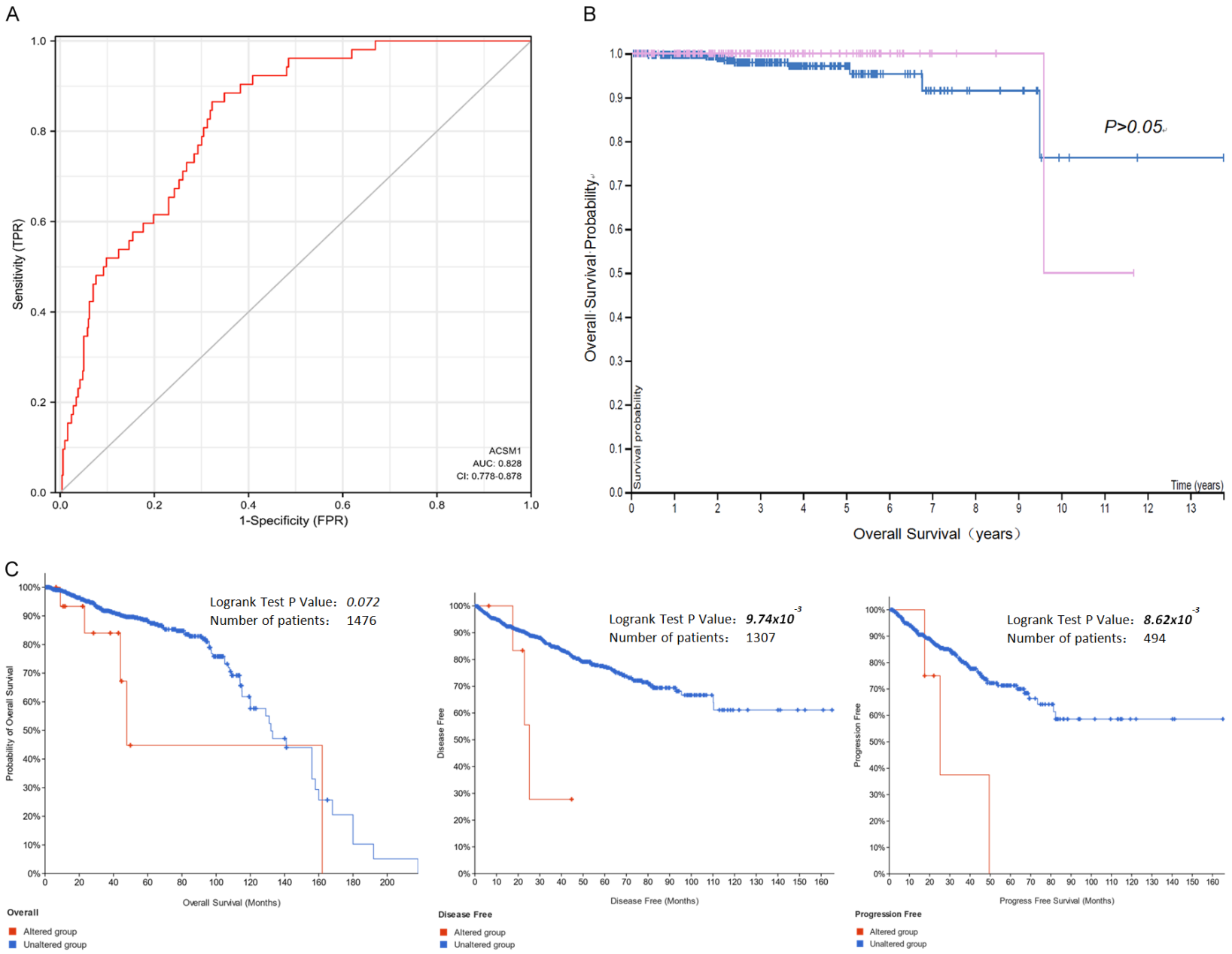

A
Sensitivity (TPR)
1-Specificity (FPR)
ACSM1
AUC: 0.828
CI: 0.778-0.878
B
Overall Survival Probability
Survival probability
P>0.05
Time (years)
Overall Survival（years）
C
Logrank Test P Value: 0.072
Number of patients: 1476
Probability of Overall Survival
Overall Survival (Months)
Overall
Altered group
Unaltered group
Logrank Test P Value: 9.74x10-3
Number of patients: 1307
Disease Free
Disease Free (Months)
Logrank Test P Value: 8.62x10-3
Number of patients: 494
Progression Free
Progress Free Survival (Months)

Figure 4. Prognostic significance of ACSM1 in prostate cancer. A. ACSM1 expression increased the risk of prostate cancer progression. Area Under Curve (AUC) =0.828, CI=0.778-0.878. B. ACSM1 high expression showed a trend of shorter survival in prostate cancer from TCGA prostate data from the Human Protein Atlas (https://www.proteinatlas.org). C. Genetic alterations of ACSM1 gene were significantly associated with disease-free survival and progress free survival but were not associated with overall survival (data analysis using the prostate cancer from www.cbioportal.org).

of CHD1, and overexpression of SPINK1. To evaluate the roles of ACSM1 in molecular classification of prostate cancer, an association between ACSM1 expression and molecular subtypes was analyzed. Unfortunately, the expression of ACSM1 did not show significant association with prostate cancer molecular classification (Data not shown).

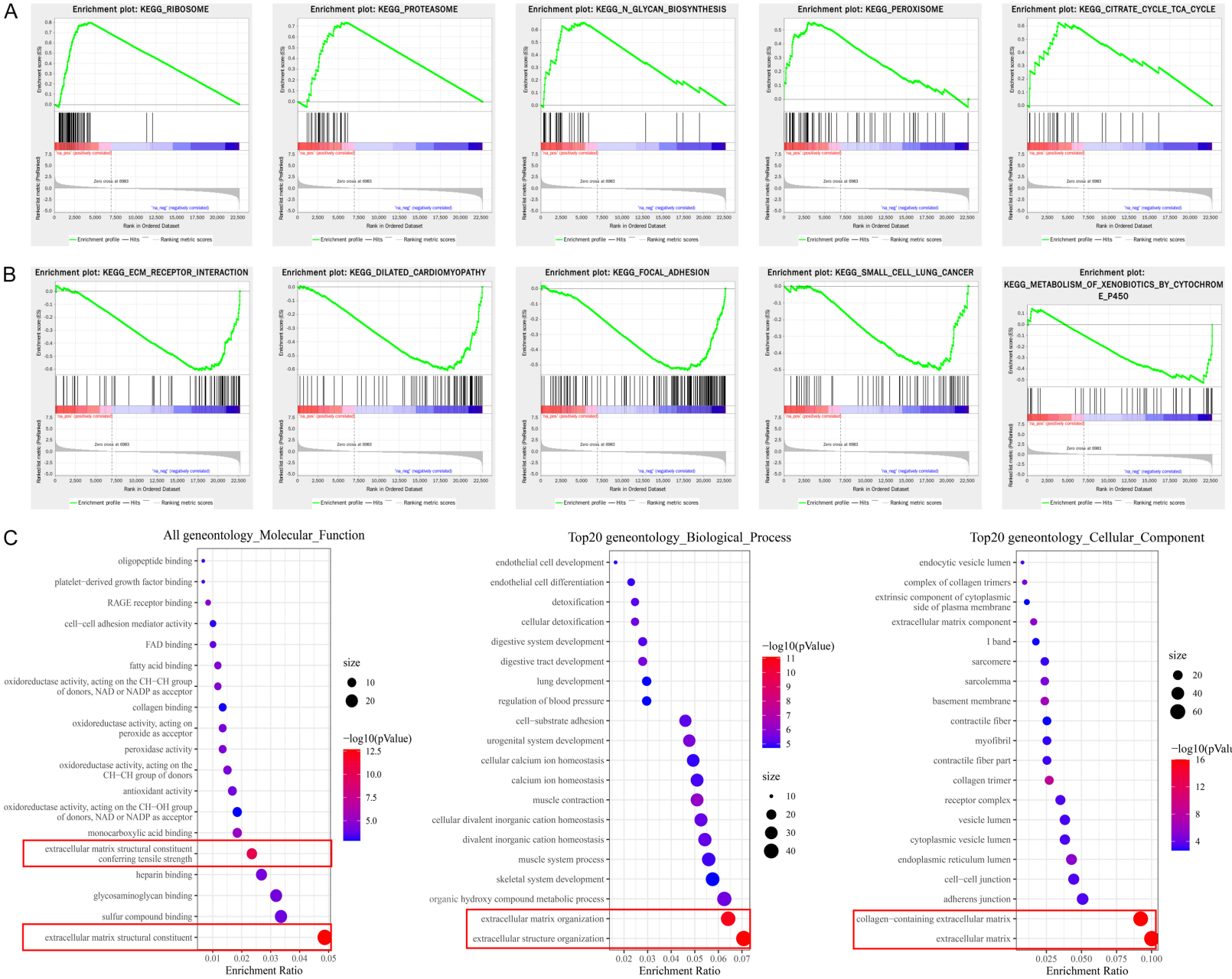
Discussion

Clinical and experimental studies have revealed numerous genes and associated-interactive signaling pathways in promoting prostate cancer development and progression [43-46]. However, biomarkers for early diagnosis and targets for therapy are rare, contributing to prostate cancer being a leader among cancer incidences and mortalities in the world. Using comprehensive approaches, we analyzed in depth gene expression profiles and genomic datasets in combination with clinical outcomes, and identified ACSM1 whose expression levels are specifically and significantly increased in prostate cancers among the 33 types of malignancies, in comparison to non-tumor prostate tissues.

PSA has been used as a specific biomarker for prostate cancer screening and for monitoring cancer recurrence and response to treatment [15, 16, 18, 19]. But concern has been raised for its inconsistency with clinical characteristics and Gleason scores. For example, some low-grade cases show higher PSA levels while high-grade cases show lower PSA levels, and some metastatic cases even show opposite PSA values. Herein we discovered that ACSM1 expression levels are highest in prostate cancer among the 33 cancer types, and significantly upregulated in cancers compared to the non-cancer prostate tissues. This is consistent with recent reports showing that ACSM1 expression levels were significantly upregulated in prostate cancer tissues at both mRNA and protein levels [47-49]. To determine the biological functions of ACSM1 in prostate cancer, Alinezhad *et al.* introduced small interfering RNAs (siRNAs) targeting human ACSM1 into prostate cancer cells and found that silencing ACSM1 resulted in marked growth arrest and cytotoxicity in PC3 and VCaP cells in 2D culture and in reduced tumor cell invasion in PC3 organoid cultures [49]. siRNA-induced prostate cancer cell growth arrest was also observed by Shrestha *et al* [47, 48]. Taken together, all the findings suggest oncogenic roles of ACSM1. Therefore, ACSM1 may be a novel biomarker for prostate cancer screening and diagnosis, metastasis monitoring, and outcome prediction. This study is the first to identify the clinical value of ACSM1 in prostate cancer, and provide additional evidence that ACSM1 may be targets for personalized therapy. However, the expression levels of ACSM1 in peripheral blood plasma and other bodily fluid remain unknown and need to be further investigated.

Recent epidemiological studies have shown the association between genomic alterations and metastasis, particularly DNA copy number variants or alterations in certain gene deletions, amplifications, or mutations in tumor tissues that had strong association with prostate cancer progression [50-52]. Dr. Sawyers has shown that CNVs can predict prostate cancer relapse [53]. Combining 6,082 non-metastatic prostate cancer cases and 1,079 metastatic prostate cancer cases from the cBioPortal dataset, we found that genomic amplifications of ACSM1 significantly increased the risk of metastasis. Thus, somatic DNA amplifications of ACSM1 will have great impact on predicting cancer metastasis and outcomes.

Changes in metabolic signaling pathways are one of the hallmarks of cancer [54] and a major cause of prostate cancer development, but no specific essential genes in this pathway have been identified or used as biomarkers in clinical settings [55-57]. Through gene function stratification and GSEA, we identified ACSM1 as an important element, whose expression and biological functions are enriched and associated with multiple signaling pathways, most of which are positively correlated with metabol-


A
Enrichment plot: KEGG_RIBOSOME
Enrichment plot: KEGG_PROTEASOME
Enrichment plot: KEGG_N_GLYCAN_BIOSYNTHESIS
Enrichment plot: KEGG_PEROXISOME
Enrichment plot: KEGG_CITRATE_CYCLE_TCA_CYCLE
B
Enrichment plot: KEGG_ECM_RECEPTOR_INTERACTION
Enrichment plot: KEGG_DILATED_CARDIOMYOPATHY
Enrichment plot: KEGG_FOCAL_ADHESION
Enrichment plot: KEGG_SMALL_CELL_LUNG_CANCER
Enrichment plot: KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450
C
All geneontology_Molecular_Function
oligopeptide binding
platelet−derived growth factor binding
RAGE receptor binding
cell−cell adhesion mediator activity
FAD binding
fatty acid binding
oxidoreductase activity, acting on the CH−CH group of donors, NAD or NADP as acceptor
collagen binding
oxidoreductase activity, acting on peroxide as acceptor
peroxidase activity
oxidoreductase activity, acting on the CH−CH group of donors
antioxidant activity
oxidoreductase activity, acting on the CH−OH group of donors, NAD or NADP as acceptor
monocarboxylic acid binding
extracellular matrix structural constituent conferring tensile strength
heparin binding
glycosaminoglycan binding
sulfur compound binding
extracellular matrix structural constituent
size
10
20
−log10(pValue)
12.5
10.0
7.5
5.0
0.01 0.02 0.03 0.04 0.05
Enrichment Ratio
Top20 geneontology_Biological_Process
endothelial cell development
endothelial cell differentiation
detoxification
cellular detoxification
digestive system development
digestive tract development
lung development
regulation of blood pressure
cell−substrate adhesion
urogenital system development
cellular calcium ion homeostasis
calcium ion homeostasis
muscle contraction
cellular divalent inorganic cation homeostasis
divalent inorganic cation homeostasis
muscle system process
skeletal system development
organic hydroxy compound metabolic process
extracellular matrix organization
extracellular structure organization
−log10(pValue)
11
10
9
8
7
6
5
size
10
20
30
40
0.02 0.03 0.04 0.05 0.06 0.07
Enrichment Ratio
Top20 geneontology_Cellular_Component
endocytic vesicle lumen
complex of collagen trimers
extrinsic component of cytoplasmic side of plasma membrane
extracellular matrix component
I band
sarcomere
sarcolemma
basement membrane
contractile fiber
myofibril
contractile fiber part
collagen trimer
receptor complex
vesicle lumen
cytoplasmic vesicle lumen
endoplasmic reticulum lumen
cell−cell junction
adherens junction
collagen−containing extracellular matrix
extracellular matrix
size
20
40
60
−log10(pValue)
16
12
8
4
0.025 0.050 0.075 0.100
Enrichment Ratio

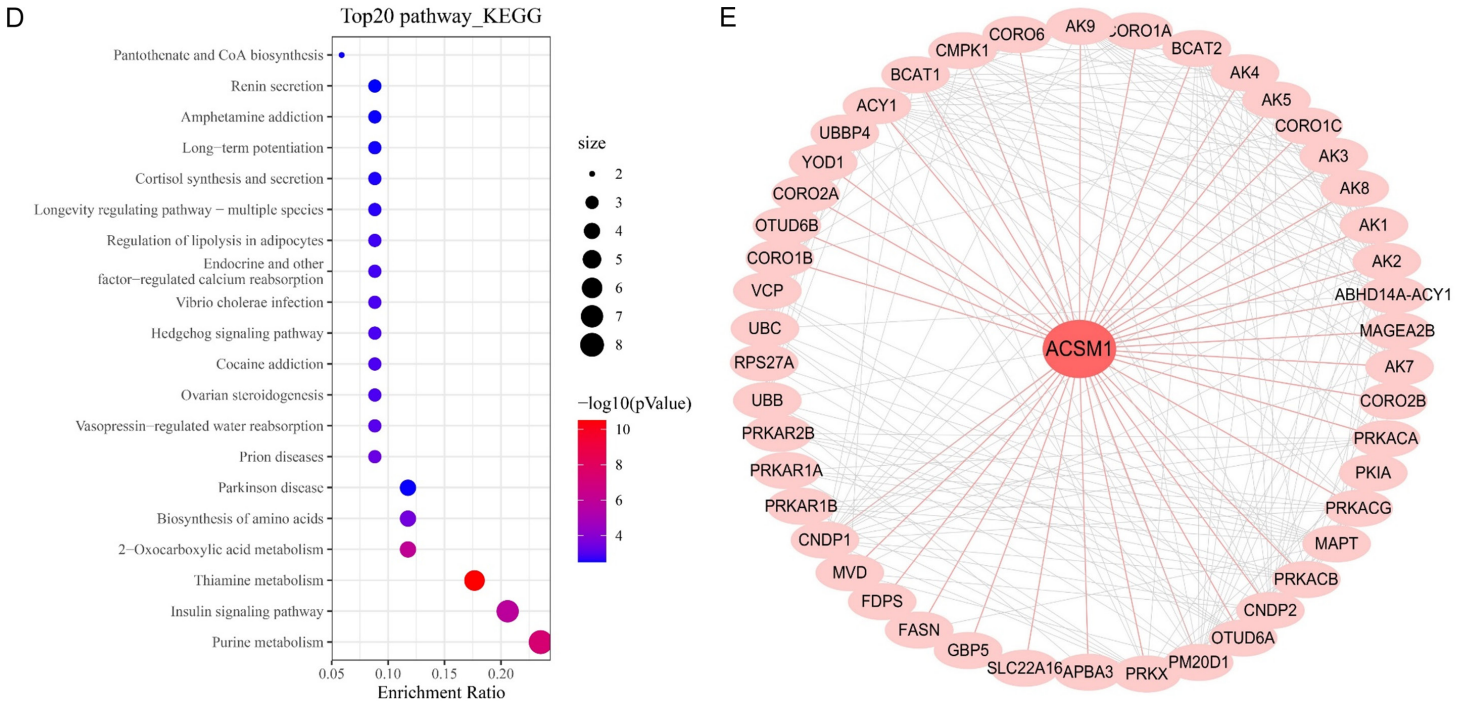


Figure 5. Gene set enrichment analysis and GO/KEGG analysis revealed ACSM1 gene-associated signaling pathways in prostate cancer and in pan-cancer. A. Top 5 up-regulated gene sets (positively enriched) on the Rank Ordered List by GESA: Ribosome, Proteasome, N Glycan Biosynthesis, Peroxisome, and Citrate TCA Cycle signaling pathways. B. TOP 5 down-regulated gene sets (negatively enriched) on the Rank Ordered List by GSEA: ECM Receptor Interaction, Dilated Cardiomyopathy, Focal Adhesion, Small Cell Lung cancer, and Metabolism of Xenobiotics by Cytochrome P450 signaling pathways. C. Significantly enriched GO terms of differentially expressed genes in prostate cancer from TCGA database. GO analysis classified the DEGs into 3 groups (i.e. Cellular Component, Biological Process, and Molecular Function), in which, extracellular matrix and extracellular-associated signaling pathways were significantly involved in ACSM1-associated molecular functions, biological process, and cellular components (Shown in red box). D. The KEGG pathways of ACSM1-associated genes. Top 20 pathways were shown. E. ACSM1-associated genes protein-protein interaction (PPI) network complex analysis. The modules of ACSM1 associated genes was filtered with MCODE. Among them, 47 ACSM1-associated genes were displayed.

ic signaling pathways. Numerous studies have implicated metabolic and inflammatory pathways in prostate cancer development. Herein we found that critical metabolic signaling such as Butanoate metabolism, Citrate TCA Cycle signaling, Peroxisome, Amino sugar and Nucleotide Sugar Metabolism, and Protein Export, Valine Leucine and Isoleucine Degradation, Ribosome, Proteasome, and N Glycan Biosynthesis are positively enriched with ACSM1 in prostate cancer and in pan-cancer. In fact, an *in vitro* study showed that knockdown of the ACSM1 gene resulted in ATP depletion in prostate cancer cells. Cellular lipidomic analysis showed that polyunsaturated fatty acids accumulated with the reduction of the ACSM1 gene. Metabolomics revealed that cells adapted to ACSM1 reduction through glycolysis. Moreover, knockdown of the ACSM1 gene led to metabolic dysregulation, mitochondrial oxidative stress, and lipid peroxidation, ultimately leading to cell death [47, 48]. The involvement of the ACSM1 gene in metabolic signaling is also closely related to its upstream polymerase-delta-interacting protein 2 (Poldip2). Several studies have shown that Poldip2 deficiency leads to ACSM1 degradation. Poldip2 is a nuclear-encoded mitochondrial protein that has been confirmed to regulate fatty acylation through caseinolytic peptidase (Clp) complex-mediated ACSM1 degradation [5, 58-60]. These studies collectively provide direct evidence that demonstrates the critical roles of ACSM1 in metabolism.

Ribosome biogenesis is a fundamental cellular process linked to cell growth and proliferation. Ribosome biogenesis and signaling are upregulated in most of cancers, usually enhanced by MYC in prostate cancer, which in turn further stimulates prostate cancer growth and progression [61-63]. Therefore, in recent years targeting the ribosome has become a new therapeutic approach to treat advanced prostate cancer [64, 65]. Studying these signaling pathways will improve our understanding of the development and progression of prostate cancer, and also provide critical information for developing novel adjuvant therapies for prostate cancer.

Cancer progression, recurrence, and metastasis are highly associated with microenvironmental factors, in which the extracellular matrix (ECM) is one of the key components. ECM is a non-cellular three-dimensional macromolecular modeling system and a highly dynamic structural network of collagens, proteoglycans, glycoproteins, fibronectin, laminins, elastin, immune cells, growth factors, cytokines and chemokines, and other elements. Matrix elements bind each other as well as cell adhesion receptors via complicated signaling systems to form a complex network that controls organ and tissue residual cells and physical functions. Cell surface receptors transduce ECM signals into cells and regulate cell differentiation, proliferation, migration, death, and survival, and play a critical role in maintaining cellular homeostasis. Deregulation of ECM composition and structure is associated with carcinogenesis and cancer progression [66]. In this study, GSEA also identified that ACSM1 expression was negatively correlated with ECM-receptor interaction pathway, one of the critical pathways contributing to cancer formation, progression, metastasis, and responses to cancer treatment. In addition, gene functional stratification analysis further showed significantly enriched GO terms of differentially expressed genes (DEGs) from prostate cancer, from which Molecular Function, Biological Process and Cellular Component groups were classified. In all three groups, the significantly changed and the highest number of genes changed were extracellular matrix (ECM)-associated gene sets, including extracellular matrix structural constituent and extracellular matrix structural constituent conferring tensile strength in Molecular Function Group; extracellular matrix organization and extracellular structure organization in the Biological Process group; and finally, extracellular and collagen-containing extracellular matrix genes in the Cellular Component group. These observations indicate that ECM-receptor interaction pathway and deregulation of ECM composition and structure may be involved in the development and metastasis of prostate cancer.

We also found Gap Junction signaling and Tight Junction signaling in this ECM-receptor interaction pathway, both of which are key elements in cancer cell integrity maintenance and cell adhesion [67-71]. Therefore, it is hypothesized that the upregulation of ACSM1 is negatively correlated with extracellular matrix (ECM) and ECM receptors to facilitate cancer metas-

tasis, and that ACSM1 could be a potential therapeutic target [68, 69, 72].

Consistent with the enrichment analysis, GO functional stratification showed that similar molecular functions of ACSM1 were specifically enriched in prostate cancer (**Figure 5C**) and in pan-cancers, which was further confirmed by KEGG analysis where most signaling pathways are related to metabolism and extracellular matrix components (**Figure 5D**). Again, Protein-protein interaction exhibited ACSM1-correlated interaction at protein levels (**Figure 5E**).

Immune functions have been known as one of the key elements affecting cancer formation and outcomes. Cancer immunotherapy has revolutionized cancer care, and antibodies against CTLA-4, PD-1, and PD-L1 have proven effective in treating a variety of malignancies [73, 74]. Therefore, to characterize the cancer immune microenvironment is critical for understanding the mechanisms of cancer progression and designing immunotherapy strategies. Using Tumor Immune Estimating Resource (TIMER) (https://cistrome.shinyapps.io/timer/) [31], we evaluated the association between ACSM1 expression and immune cell infiltration in cancer environment. Unexpected, no association was observed between ACSM1 expression and the infiltration of B cells, CD8+/CD4+ T cells, neutrophils, macrophages, or dendritic cells. In addition, ACSM1 expression was not correlated with any immune subtypes of prostate cancer.

Molecular classifications have been widely used in precision oncology for cancer outcome prediction and personalized treatment [75, 76]. Based on molecular alterations, prostate cancer has been classified into 7 major molecular subtypes (4 gene fusions and 3 gene recurrent mutations) [33-35, 77, 78]. Our stratification analysis did not identify a significant association between ACSM1 expression and molecular classification in prostate cancer, more samples from other studies are sorely needed for further investigation into these questions before more definitive conclusions can be made.

Conclusions

The present work has demonstrated that ACSM1 is specifically and significantly upregulated in prostate cancer, ACSM1 gene expression and genomic amplification exhibit clinical significance through metabolic and ECM-receptor interaction signaling pathways. Thus, ACSM1 may be a novel oncogene and serve as a biomarker for prostate cancer screening, prognosis prediction, and as a therapeutic target. This work promises to have great impact on personalized prevention and therapy for prostate cancer.

Acknowledgements

This project was supported in part by Doctoral Research Startup Fund from Mudanjiang Medical University (2021-MYBSKY-064 to Bao Y and 2021-MYBSKY-065 to Guo Y).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yonghua Bao, Department of Pathology, Mudanjiang Medical University, Mudanjiang 157011, China. Tel: +86-453-6982060; E-mail: baoyonghua@mdjmu.edu.cn; Dr. Wancai Yang, Department of Pathology, University of Illinois at Chicago, IL 60612, USA. Tel: 1-630-2101819; E-mail: wyang06@uic.edu

References

[1] Fujino T, Takei YA, Sone H, Ioka RX, Kamataki A, Magoori K, Takahashi S, Sakai J and Yamamoto TT. Molecular identification and characterization of two medium-chain acyl-CoA synthetases, MACS1 and the Sa gene product. J Biol Chem 2001; 276: 35961-35966.

[2] Vessey DA, Kelley M and Warren RS. Characterization of the CoA ligases of human liver mitochondria catalyzing the activation of short- and medium-chain fatty acids and xenobiotic carboxylic acids. Biochim Biophys Acta 1999; 1428: 455-462.

[3] Boomgaarden I, Vock C, Klapper M and Doring F. Comparative analyses of disease risk genes belonging to the acyl-CoA synthetase medium-chain (ACSM) family in human liver and cell lines. Biochem Genet 2009; 47: 739-748.

[4] van der Sluis R and Erasmus E. Xenobiotic/medium chain fatty acid: CoA ligase - a critical review on its role in fatty acid metabolism and the detoxification of benzoic acid and aspirin. Expert Opin Drug Metab Toxicol 2016; 12: 1169-1179.

[5] Paredes F, Sheldon K, Lassegue B, Williams HC, Faidley EA, Benavides GA, Torres G, San-

hueza-Olivares F, Yeligar SM, Griendling KK, Darley-Usmar V and San Martin A. Poldip2 is an oxygen-sensitive protein that controls PDH and alphaKGDH lipoylation and activation to support metabolic adaptation in hypoxia and cancer. Proc Natl Acad Sci U S A 2018; 115: 1789-1794.

[6] Bailey PSJ, Hiltunen JK, Dieckmann CL, Kastaniotis AJ and Nathan JA. Different opinion on the reported role of Poldip2 and ACSM1 in a mammalian lipoic acid salvage pathway controlling HIF-1 activation. Proc Natl Acad Sci U S A 2018; 115: E7458-E7459.

[7] Li W, Ji W, Li Z, He K, Wang Q, Chen J, Qiang Y, Feng G, Li X, Shen J, Wen Z, Ji J and Shi Y. Genetic association of ACSM1 variation with schizophrenia and major depressive disorder in the Han Chinese population. Am J Med Genet B Neuropsychiatr Genet 2015; 168B: 144-149.

[8] Celis JE, Gromov P, Cabezon T, Moreira JM, Friis E, Jirstrom K, Llombart-Bosch A, Timmermans-Wielenga V, Rank F and Gromova I. 15-prostaglandin dehydrogenase expression alone or in combination with ACSM1 defines a subgroup of the apocrine molecular subtype of breast carcinoma. Mol Cell Proteomics 2008; 7: 1795-1809.

[9] Azad RK and Shulaev V. Metabolomics technology and bioinformatics for precision medicine. Brief Bioinform 2019; 20: 1957-1971.

[10] Wanichthanarak K, Fahrmann JF and Grapov D. Genomic, proteomic, and metabolomic data integration strategies. Biomark Insights 2015; 10: 1-6.

[11] Rajesh S, Cox MJ and Runau F. Molecular advances in pancreatic cancer: a genomic, proteomic and metabolomic approach. World J Gastroenterol 2021; 27: 5171-5180.

[12] Hong M, Tao S, Zhang L, Diao LT, Huang X, Huang S, Xie SJ, Xiao ZD and Zhang H. RNA sequencing: new technologies and applications in cancer research. J Hematol Oncol 2020; 13: 166.

[13] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022; 72: 7-33.

[14] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2021. CA Cancer J Clin 2021; 71: 7-33.

[15] Albertsen PC. Prostate-specific antigen screening using the traditional cut point of 3 ng/ml: Con. Eur Urol Focus 2021; 7: 501-502.

[16] Albertsen PC. PSA testing, cancer treatment, and prostate cancer mortality reduction: what is the mechanism? Urol Oncol 2021; In press.

[17] Albertsen PC. Prostate cancer screening: a new way forward or another false start? Nat Rev Urol 2021; 18: 579-580.

[18] Sopyllo K, Erickson AM and Mirtti T. Grading evolution and contemporary prognostic biomarkers of clinically significant prostate cancer. Cancers (Basel) 2021; 13: 628.

[19] Gao Z, Pang B, Li J, Gao N, Fan T and Li Y. Emerging role of exosomes in liquid biopsy for monitoring prostate cancer invasion and metastasis. Front Cell Dev Biol 2021; 9: 679527.

[20] Wilt TJ, Ullman KE, Linskens EJ, MacDonald R, Brasure M, Ester E, Nelson VA, Saha J, Sultan S and Dahm P. Therapies for clinically localized prostate cancer: a comparative effectiveness review. J Urol 2021; 205: 967-976.

[21] Singh S, Moore CM, Punwani S, Mitra AV and Bandula S. Long-term biopsy outcomes in prostate cancer patients treated with external beam radiotherapy: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis 2021; 24: 612-622.

[22] Nuhn P, De Bono JS, Fizazi K, Freedland SJ, Grilli M, Kantoff PW, Sonpavde G, Sternberg CN, Yegnasubramanian S and Antonarakis ES. Update on systemic prostate cancer therapies: management of metastatic castration-resistant prostate cancer in the era of precision oncology. Eur Urol 2019; 75: 88-99.

[23] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.

[24] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.

[25] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.

[26] Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D and Groop LC. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003; 34: 267-273.

[27] Wang J, Vasaikar S, Shi Z, Greer M and Zhang B. WebGestalt 2017: a more comprehensive, powerful, flexible and interactive gene set enrichment analysis toolkit. Nucleic Acids Res 2017; 45: W130-W137.

[28] Yu G, Wang LG, Han Y and He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012; 16: 284-287.
[29] Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C and Jensen LJ. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013; 41: D808-815.
[30] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
[31] Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B and Liu XS. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017; 77: e108-e110.
[32] Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, Chu KC, Wong CY, Lau CY, Chen I, Chan NW and Zhang J. TISIDB: an integrated repository portal for tumor-immune system interactions. Bioinformatics 2019; 35: 4200-4202.
[33] Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015; 163: 1011-1025.
[34] Kaffenberger SD and Barbieri CE. Molecular subtyping of prostate cancer. Curr Opin Urol 2016; 26: 213-218.
[35] Santoni M, Cimadamore A, Massari F, Sorgentoni G, Cheng L, Lopez-Beltran A, Battelli N and Montironi R. Narrative review: predicting future molecular and clinical profiles of prostate cancer in the United States. Transl Androl Urol 2021; 10: 1562-1568.
[36] Arredouani MS, Lu B, Bhasin M, Eljanne M, Yue W, Mosquera JM, Bubley GJ, Li V, Rubin MA, Libermann TA and Sanda MG. Identification of the transcription factor single-minded homologue 2 as a potential biomarker and immunotherapy target in prostate cancer. Clin Cancer Res 2009; 15: 5794-5802.
[37] Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M, Mehra R, Prensner JR, Palanisamy N, Ryslik GA, Vandin F, Raphael BJ, Kunju LP, Rhodes DR, Pienta KJ, Chinnaiyan AM and Tomlins SA. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012; 487: 239-243.
[38] Liu P, Ramachandran S, Ali Seyed M, Scharer CD, Laycock N, Dalton WB, Williams H, Karanam S, Datta MW, Jaye DL and Moreno CS. Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells. Cancer Res 2006; 66: 4011-4019.
[39] Luo JH, Yu YP, Cieply K, Lin F, Deflavia P, Dhir R, Finkelstein S, Michalopoulos G and Becich M. Gene expression analysis of prostate cancers. Mol Carcinog 2002; 33: 25-35.
[40] Vanaja DK, Cheville JC, Iturria SJ and Young CY. Transcriptional silencing of zinc finger protein 185 identified by expression profiling is associated with prostate cancer progression. Cancer Res 2003; 63: 3877-3882.
[41] Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson HF Jr and Hampton GM. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res 2001; 61: 5974-5978.
[42] Holzbeierlein J, Lal P, LaTulippe E, Smith A, Satagopan J, Zhang L, Ryan C, Smith S, Scher H, Scardino P, Reuter V and Gerald WL. Gene expression analysis of human prostate carcinoma during hormonal therapy identifies androgen-responsive genes and mechanisms of therapy resistance. Am J Pathol 2004; 164: 217-227.
[43] Pisano C, Tucci M, Di Stefano RF, Turco F, Scagliotti GV, Di Maio M and Buttigliero C. Interactions between androgen receptor signaling and other molecular pathways in prostate cancer progression: current and future clinical implications. Crit Rev Oncol Hematol 2021; 157: 103185.
[44] Aurilio G, Cimadamore A, Mazzucchelli R, Lopez-Beltran A, Verri E, Scarpelli M, Massari F, Cheng L, Santoni M and Montironi R. Androgen receptor signaling pathway in prostate cancer: from genetics to clinical applications. Cells 2020; 9: 2653.
[45] Bluemn EG, Coleman IM, Lucas JM, Coleman RT, Hernandez-Lopez S, Tharakan R, Bianchi-Frias D, Dumpit RF, Kaipainen A, Corella AN, Yang YC, Nyquist MD, Mostaghel E, Hsieh AC, Zhang X, Corey E, Brown LG, Nguyen HM, Pienta K, Ittmann M, Schweizer M, True LD, Wise D, Rennie PS, Vessella RL, Morrissey C and Nelson PS. Androgen receptor pathway-independent prostate cancer is sustained through FGF signaling. Cancer Cell 2017; 32: 474-489, e476.
[46] Attard G, Richards J and de Bono JS. New strategies in metastatic prostate cancer: targeting the androgen receptor signaling pathway. Clin Cancer Res 2011; 17: 1649-1657.
[47] Shrestha RK. Elucidating the molecular mechanisms underlying androgen-regulated lipid metabolism in prostate cancer. Digital.library.adelaide.edu.au 2020.
[48] Shrestha RK, Townley S, Hanson A, Pickering M, Nassar ZD, Mah CY, Ghodsi MA, Hoy AJ, Quek LE, Tilley WD, Butler LM and Selth LA. Abstract PO-036: ACSM1 and ACSM3 regulate

fatty acid oxidation in prostate cancer to promote growth and protect against oxidative stress. Cancer Res 2020; 80: P0-036-P0-036.
[49] Alinezhad S, Vaananen RM, Mattsson J, Li Y, Tallgren T, Tong Ochoa N, Bjartell A, Akerfelt M, Taimen P, Bostrom PJ, Pettersson K and Nees M. Validation of novel biomarkers for prostate cancer progression by the combination of bioinformatics, clinical and functional studies. PLoS One 2016; 11: e0155901.
[50] Liu W. DNA alterations in the tumor genome and their associations with clinical outcome in prostate cancer. Asian J Androl 2016; 18: 533-542.
[51] Liu W, Wang L and Xu J. Somatic DNA copy number alterations and their potential clinical utility for predicting lethal prostate cancer. Asian J Androl 2013; 15: 586-587.
[52] Liu W, Hou J, Petkewicz J, Na R, Wang CH, Sun J, Gallagher J, Bogachkov YY, Swenson L, Regner M, Resurreccion WK, Isaacs WB, Brendler CB, Crawford S, Zheng SL, Helfand BT and Xu J. Feasibility and performance of a novel probe panel to detect somatic DNA copy number alterations in clinical specimens for predicting prostate cancer progression. Prostate 2020; 80: 1253-1262.
[53] Hieronymus H, Schultz N, Gopalan A, Carver BS, Chang MT, Xiao Y, Heguy A, Huberman K, Bernstein M, Assel M, Murali R, Vickers A, Scardino PT, Sander C, Reuter V, Taylor BS and Sawyers CL. Copy number alteration burden predicts prostate cancer relapse. Proc Natl Acad Sci U S A 2014; 111: 11139-11144.
[54] Hanahan D. Hallmarks of cancer: new dimensions. Cancer Discov 2022; 12: 31-46.
[55] Bader DA and McGuire SE. Tumour metabolism and its unique properties in prostate adenocarcinoma. Nat Rev Urol 2020; 17: 214-231.
[56] Tewari AK, Stockert JA, Yadav SS, Yadav KK and Khan I. Inflammation and prostate cancer. Adv Exp Med Biol 2018; 1095: 41-65.
[57] Staal J and Beyaert R. Inflammation and NF-kappaB signaling in prostate cancer: mechanisms and clinical implications. Cells 2018; 7: 122.
[58] Golias T, Kery M, Radenkovic S and Papandreou I. Microenvironmental control of glucose metabolism in tumors by regulation of pyruvate dehydrogenase. Int J Cancer 2019; 144: 674-686.
[59] Paredes F, Williams H and San Martin A. Poldip2 is an oxygen-sensitive mitochondrial protein that controls oxidative/glycolytic metabolism balance and proteasome activity. Free Radic Biol Med 2017; 112: 173-174.
[60] Paredes F, Williams HC, Quintana RA and San Martin A. Mitochondrial protein poldip2 (Polymerase Delta Interacting Protein 2) controls vascular smooth muscle differentiated phenotype by O-Linked GlcNAc (N-Acetylglucosamine) transferase-dependent inhibition of a ubiquitin proteasome system. Circ Res 2020; 126: 41-56.
[61] Fenner A. Prostate cancer: targeting the ribosome in advanced disease. Nat Rev Urol 2016; 13: 562.
[62] Iglesias-Gato D, Wikstrom P, Tyanova S, Lavallee C, Thysell E, Carlsson J, Hagglof C, Cox J, Andren O, Stattin P, Egevad L, Widmark A, Bjartell A, Collins CC, Bergh A, Geiger T, Mann M and Flores-Morales A. The proteome of primary prostate cancer. Eur Urol 2016; 69: 942-952.
[63] Ray S, Johnston R, Campbell DC, Nugent S, McDade SS, Waugh D and Panov KI. Androgens and estrogens stimulate ribosome biogenesis in prostate and breast cancer cells in receptor dependent manner. Gene 2013; 526: 46-53.
[64] Lawrence MG, Obinata D, Sandhu S, Selth LA, Wong SQ, Porter LH, Lister N, Pook D, Pezaro CJ, Goode DL, Rebello RJ, Clark AK, Papargiris M, Van Gramberg J, Hanson AR, Banks P, Wang H, Niranjan B, Keerthikumar S, Hedwards S, Huglo A, Yang R, Henzler C, Li Y, Lopez-Campos F, Castro E, Toivanen R, Azad A, Bolton D, Goad J, Grummet J, Harewood L, Kourambas J, Lawrentschuk N, Moon D, Murphy DG, Sengupta S, Snow R, Thorne H, Mitchell C, Pedersen J, Clouston D, Norden S, Ryan A, Dehm SM, Tilley WD, Pearson RB, Hannan RD, Frydenberg M, Furic L, Taylor RA and Risbridger GP. Patient-derived models of abiraterone- and enzalutamide-resistant prostate cancer reveal sensitivity to ribosome-directed therapy. Eur Urol 2018; 74: 562-572.
[65] Rebello RJ, Kusnadi E, Cameron DP, Pearson HB, Lesmana A, Devlin JR, Drygin D, Clark AK, Porter L, Pedersen J, Sandhu S, Risbridger GP, Pearson RB, Hannan RD and Furic L. The dual inhibition of RNA Pol I transcription and PIM kinase as a new therapeutic approach to treat advanced prostate cancer. Clin Cancer Res 2016; 22: 5539-5552.
[66] Theocharis AD, Skandalis SS, Gialeli C and Karamanos NK. Extracellular matrix structure. Adv Drug Deliv Rev 2016; 97: 4-27.
[67] Moustakas A and Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci 2007; 98: 1512-1520.
[68] Mulkearns-Hubert EE, Reizes O and Lathia JD. Connexins in cancer: jekyll or hyde? Biomolecules 2020; 10: 1654.
[69] Chen CX, Luo KJ, Yang JP, Huang YC, Cardenas ER, Nicholson BJ and Jiang JX. Connexins and cAMP cross-talk in cancer progression and metastasis. Cancers (Basel) 2020; 13: 58.

[70] Bhat AA, Uppada S, Achkar IW, Hashem S, Yadav SK, Shanmugakonar M, Al-Naemi HA, Haris M and Uddin S. Tight junction proteins and signaling pathways in cancer and inflammation: a functional crosstalk. Front Physiol 2018; 9: 1942.

[71] Kyuno D, Takasawa A, Kikuchi S, Takemasa I, Osanai M and Kojima T. Role of tight junctions in the epithelial-to-mesenchymal transition of cancer cells. Biochim Biophys Acta Biomembr 2021; 1863: 183503.

[72] Liu M, Yang J, Xu B and Zhang X. Tumor metastasis: mechanistic insights and therapeutic interventions. MedComm (2020) 2021; 2: 587-617.

[73] Meng J, Zhou Y, Lu X, Bian Z, Chen Y, Zhou J, Zhang L, Hao Z, Zhang M and Liang C. Immune response drives outcomes in prostate cancer: implications for immunotherapy. Mol Oncol 2021; 15: 1358-1375.

[74] Ozdemir BC, Siefker-Radtke AO, Campbell MT and Subudhi SK. Current and future applications of novel immunotherapies in urological oncology: a critical review of the literature. Eur Urol Focus 2018; 4: 442-454.

[75] Nevedomskaya E, Baumgart SJ and Haendler B. Recent advances in prostate cancer treatment and drug discovery. Int J Mol Sci 2018; 19: 1359.

[76] Jairath NK, Dal Pra A, Vince R Jr, Dess RT, Jackson WC, Tosoian JJ, McBride SM, Zhao SG, Berlin A, Mahal BA, Kishan AU, Den RB, Freedland SJ, Salami SS, Kaffenberger SD, Pollack A, Tran P, Mehra R, Morgan TM, Weiner AB, Mohamad O, Carroll PR, Cooperberg MR, Karnes RJ, Nguyen PL, Michalski JM, Tward JD, Feng FY, Schaeffer EM and Spratt DE. A systematic review of the evidence for the decipher genomic classifier in prostate cancer. Eur Urol 2021; 79: 374-383.

[77] Nicholas TR, Strittmatter BG and Hollenhorst PC. Oncogenic ETS factors in prostate cancer. Adv Exp Med Biol 2019; 1210: 409-436.

[78] Qian C, Li D and Chen Y. ETS factors in prostate cancer. Cancer Lett 2022; 530: 181-189.