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A Pan-Cancer Analysis of GAPDH as a Common Biomarker for Various Cancers

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Abstract: *Objective:* To investigate the expression levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and explore its prognostic value across 24 different human cancers. This investigation was conducted using comprehensive bioinformatics and in vitro approaches, incorporating multiple layers of analysis. *Methods:* GAPDH expression and methylation levels were assessed using bioinformatics tools and validated in cell lines through RNA-seq and targeted bisulfite-seq analyses. The potential prognostic significance of GAPDH was evaluated using the KM plotter. Additionally, cBioPortal was employed to investigate genetic alterations associated with this gene. Pathway analysis was conducted using DAVID. Furthermore, a correlation analysis between GAPDH expression and $CD8^+$ T immune cells was performed using TIMER and CDT. Finally, a gene-drug interaction network analysis was conducted using Cytoscape to examine the relationship between GAPDH and various drugs. *Results:* GAPDH was found to be commonly upregulated in 24 types of human cancers, with its upregulation significantly correlated with poor relapse-free survival (RFS) and overall survival (OS) in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. This suggests that GAPDH plays a significant role in the development of these cancers. GAPDH upregulation was also associated with various clinicopathological features in patients with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. Pathway analysis revealed GAPDH's involvement in diverse pathways. Additionally, notable correlations were observed between GAPDH expression and its promoter methylation level, genetic alterations, and $CD8^+$ T immune cell levels. Moreover, several regulatory drugs targeting GAPDH were identified, with the potential to modulate its expression and potentially prevent conditions such as BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. *Conclusion:* Based on our findings, GAPDH emerges as a promising diagnostic and prognostic biomarker for BLCA, CESC, HNSC, KIRP, LIHC, and LUAD.

Keywords: Cancer; Expression; GAPDH; Biomarker

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1. Introduction

Cancer is not a single disease but a collection of various diseases [1], characterized by the uncontrolled growth and spread of abnormal cells in the body. It can arise in any part of the body and manifest in numerous forms, such as breast, lung, prostate, and colon cancers [2]. Each type of cancer has its unique characteristics, including specific risk factors, symptoms, and treatment approaches. The impact of cancer extends beyond the physical

realm, affecting individuals emotionally, socially, and economically [3]. It places a significant burden on patients, their families, and healthcare systems. The development of cancer can be influenced by various factors, including genetic mutations, environmental exposures, lifestyle choices, and certain infections [4]. Therefore, cancer can also be described as a disorder of altered gene expression, primarily resulting from variations in the expression of various DNA repair and tumor suppressor genes [5]. According to 2019 disease prevalence and mortality statistics, cancer has been declared the second leading cause of death worldwide, with an estimated 9.6 million deaths, or one in six deaths, following cardiovascular diseases [6].

Recently, an increasing body of evidence has suggested that regulatory changes resulting in the alteration of gene expression play a critical role in complex traits and disorders, and such genomic changes with regulatory effects are also predicted to participate in the development of cancer [7]. Identifying these regulatory alterations and their impact on gene expression levels is crucial for understanding cancer biology. Major cancer subtypes, including breast cancer, colorectal cancer, and leukemia, have typically been profiled for CpG methylation, post-transcriptional, post-translational changes, and mutational analysis of a few DNA repair and tumor suppressor genes to understand the molecular landscape of cancer development [8]. However, the effect of such regulatory alterations on the gene expression of several other essential genes remains to be uncovered.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an important enzyme in the human body that catalyzes the redox reaction via the glycolytic pathway [9]. GAPDH is a housekeeping enzyme and is thus frequently used as an internal control in various laboratory-based experiments, including Western blotting and RT-PCR [10,11]. Earlier evidence linked GAPDH upregulation with cancer development, as initial findings were documented in the Dunning R-3327 rat prostatic adenocarcinoma, where higher expression of GAPDH was observed in cancer cells compared to the ventral prostate tissue of normal rats [12]. Additionally, Tang *et al.* [13] reported higher GAPDH expression in human colon cancer (CC) tissue compared to controls, with even higher levels in metastatic liver tissue, suggesting that GAPDH may contribute to colon cancer metastasis. In contrast, Seykora *et al.* reported that GAPDH expression might be slightly diminished in melanoma metastases and nodular primary melanomas relative to melanocytic nevi [14], while other reports have shown significant upregulation of GAPDH in melanoma tissues compared to normal tissues [15]. Furthermore, recent studies have identified point mutations in the GAPDH gene as a novel melanoma tumor antigen, recognized by tumor-infiltrating T-lymphocytes in a patient with metastatic melanoma [16].

However, the role of GAPDH as a biomarker in other human cancers has been little reported. In the current study, we analyzed the diagnostic and prognostic potential of GAPDH in 24 human cancer subtypes using a multi-layered bioinformatics and in vitro approach. The novelty of our study lies in the pan-cancer analysis of GAPDH expression across multiple cancer types. To our knowledge, such a comprehensive examination of GAPDH expression in a diverse range of cancers has not been conducted before. By performing this pan-cancer analysis, our study uncovers previously undiscovered trends and associations between GAPDH expression and different cancer types. This broader perspective allows us to identify commonalities and variations in GAPDH expression patterns across various cancers, shedding new light on its potential roles in different oncogenic processes. Therefore, our study adds a unique and valuable dimension to the existing body of research on GAPDH and its implications in cancer biology.

2. Methods

2.1. UALCAN

GAPDH pan-cancer analysis across distinct cancers was performed using the online resource UALCAN.

This archive contains raw data from TCGA cancer projects, including data on expression, methylation, and clinicopathological parameters [17]. UALCAN is based on data extracted from The Cancer Genome Atlas (TCGA) database, one of the largest and most comprehensive cancer genomics databases in the world. The UALCAN database offers researchers access to processed and normalized transcriptome sequencing data for multiple cancer types, including lung, breast, colorectal, ovarian, and others. One of the most significant benefits of UALCAN is that it bridges the gap between cancer genomics data and easy-to-use visualization and statistical computation tools. The platform provides researchers with an intuitive way to explore cancer transcriptomic data. It incorporates several analytical tools, including gene expression analysis, patient survival analysis, tumor mutation burden analysis, and more [17]. The gene expression module, a key feature of UALCAN, enables researchers to analyze gene expression levels of their genes of interest. They can compare the expression levels with normal tissue samples and among tumor subgroups. The gene expression tool also provides charts, box plots, and heat maps. For statistical analysis, UALCAN uses a student *t*-test, with a *P*-value < 0.05 considered statistically significant.

2.2. Kaplan-Meier plotter

GAPDH relapse-free survival (RFS) and overall survival (OS) in distinct cancer subtypes were evaluated using the user-friendly online tool, Kaplan-Meier (KM) Plotter [18]. KM Plotter is a data visualization tool designed to explore the survival rates of cancer patients based on their gene expression profiles. This web-based application allows researchers to create Kaplan-Meier survival plots, perform univariate and multivariate analyses, and identify genes or markers that correlate with disease prognosis. KM Plotter enables users to analyze large cohorts of cancer patients from several public databases, including TCGA and GEO, and investigate the impact of specific genes on patient outcomes. The interface offers various customization options and statistical tools to facilitate data exploration and interpretation [18]. The outcomes of KM analysis include RFS and OS duration (in weeks) with auto-selected cutoff criteria, *P*-value, and hazard ratios. A *P*-value < 0.05 was considered statistically significant.

2.3. MEXPRESS

MEXPRESS [19] was utilized in this study to evaluate the Pearson correlation between GAPDH expression and its promoter methylation levels in different cancers. This database provides researchers with multiple analytical methods to interpret RNA sequencing data and identify relevant gene expression profiles. MEXPRESS allows users to explore gene expression data related to sample types, clinical parameters, and cancer subtypes. Users can also access gene-level data, visualizations, and data exports. MEXPRESS is particularly helpful for identifying candidates for new treatments and biomarkers. This platform is free and open to the public, making it an accessible resource for cancer research. MEXPRESS offers a unique combination of user-friendliness, versatility, and reliability, helping researchers gain insights into cancer biology in a straightforward manner [19]. A *P*-value < 0.05 was considered significant.

2.4. cBioportal

In this study, genetic alterations and copy number variations (CNVs) in GAPDH, as well as their correlation with GAPDH expression levels in distinct cancer subtypes, were evaluated using the cBioPortal database [20]. cBioPortal is a comprehensive online platform for exploring multi-omics cancer data. It integrates genomic data from public cancer datasets with analysis tools to help researchers gain insights into the molecular mechanisms underlying cancer development and progression. This database provides access to multiple types of data such as

gene expression, mutations, CNVs, protein expression, and clinical information for thousands of cancer patients across numerous cancer types. Researchers can also use a range of algorithms and visualizations to find and investigate potential cancer drivers, mutations, and clinical associations [20].

2.5. Co-expressed genes, PPI network, and pathway analysis

The GEPIA database [21] was used to identify co-expressed genes with GAPDH. The STRING database [22] was utilized to construct a protein-protein interaction (PPI) network for GAPDH using default settings. Subsequently, the PPI network was visualized using Cytoscape software version 3.8.2 [23]. Additionally, pathway analysis of the GAPDH-enriched genes was carried out using the online tool DAVID [24]. DAVID is a bioinformatics software program used by researchers to identify the biological mechanisms and pathways involved in a set of genes or proteins. The tool offers a range of analytical methods, including functional annotation, gene ontology analysis, pathway analysis, and clustering analysis. Users can upload their own gene lists or use pre-existing ones from publicly available datasets. DAVID bridges the gap between raw data and biological understanding by providing a comprehensive analysis of gene expression data. A P-value < 0.05 was considered significant.

2.6. GAPDH and CD8+ T cell infiltration levels

The Spearman correlation between GAPDH expression and $CD8^+$ T immune markers in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients was analyzed using the user-friendly resource, TIMER [25]. The TIMER database includes data on 108 cancer types from the TCGA project. TIMER2 offers several functionalities, including differential gene expression analysis, survival analysis, and gene correlations. The platform provides insights into the immune microenvironment of tumors and can aid in the development of immunotherapy strategies. TIMER uses a deconvolution algorithm that integrates multiple immune cell-specific markers to estimate the abundance of immune cells [25]. A P-value <0.05 was considered significant.

2.7. Screening of GAPDH regulatory drugs

The CTD database [26] was used to identify GAPDH regulatory drugs, including both positive and negative regulators. The CTD is a valuable resource in the fields of toxicology and genomics. It is a comprehensive and curated database that integrates information on gene-disease associations, chemical-gene interactions, and environmental factors related to toxicology. The CTD database plays a crucial role in understanding complex interactions among genes, chemicals, and diseases, ultimately contributing to the development of safer and more effective strategies for risk assessment and environmental health protection. The identified drugs were later visualized using Cytoscape version 3.8.2.

3. Results

3.1. Expression level analysis of GAPDH

The UALCAN platform was used to analyze the TCGA expression profile of GAPDH in tumor samples and their corresponding normal tissues. This analysis aimed to identify any differences in GAPDH expression between tumor and normal tissues [17]. The results revealed statistically significant overexpression of GAPDH (P < 0.05) in various human cancer samples, including BLCA, CESC, HNSC, KIRP, LIHC, and LUAD when compared to normal controls (**Figure 1**).

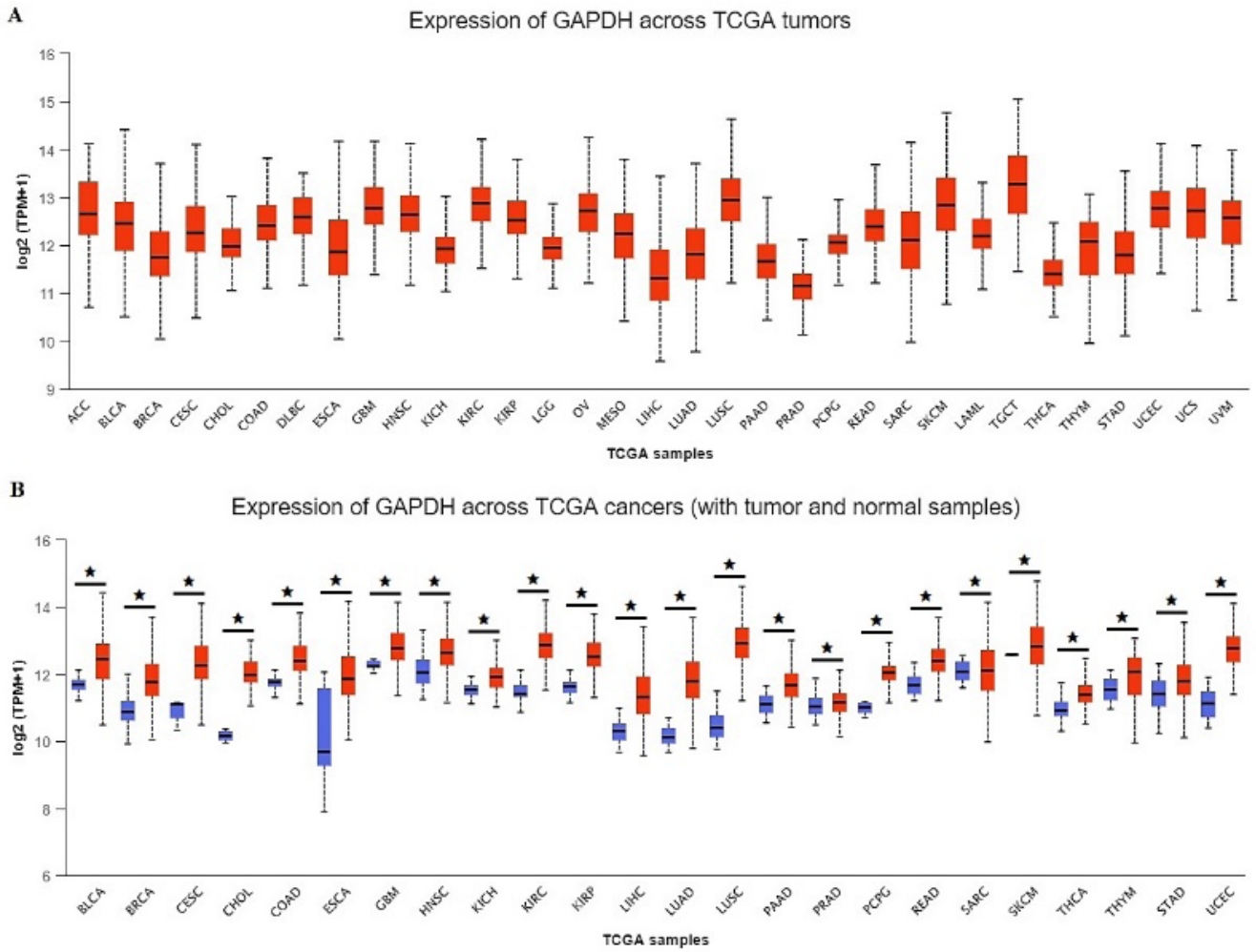

Figure 1. Profile of GAPDH expression across 24 different human cancers. **(A)** Expression profile of GAPDH in cancer samples exclusively. **(B)** Expression profile of GAPDH in both cancer samples and normal controls. A *P*-value < 0.05 indicates significance.

3.2. GAPDH prognostic potential

Prognostic analysis of GAPDH in 24 cancer types was conducted using the KM Plotter online tool. This analysis aimed to assess the relationship between GAPDH expression and prognosis, in terms of RFS and OS, across 24 cancer types. It was observed that overexpressed GAPDH was significantly associated with decreased RFS and OS duration in BLCA (HR = 1.41, 95% CI: 1.05–1.9, $P = 0.02$; HR = 1.93, 95% CI: 1.16–3.19, $P = 0.0095$), CESC (HR = 1.97, 95% CI: 1.24–3.15, $P = 0.0035$; HR = 1.81, 95% CI: 0.83–3.95, $P = 0.013$), HNSC (HR = 1.63, 95% CI: 1.17–2.29, $P = 0.0038$; HR = 1.54, 95% CI: 0.73–3.29, $P = 0.026$), KIRP (HR = 3.44, 95% CI: 2.01–6.6, $P = 5.2e^{-6}$; HR = 1.84, 95% CI: 0.82–4.14, $P = 0.013$), LIHC (HR = 2.43, 95% CI: 1.69–3.51, $P = 8.7e^{-7}$; HR = 1.75, 95% CI: 1.25–2.46, $P = 0.001$), and LUAD (HR = 1.92, 95% CI: 1.43–2.58, $P = 1.2e^{-5}$; HR = 1.55, 95% CI: 0.96–2.49, $P = 0.0069$) patients (**Figure 2**). However, in other cancer types, elevated GAPDH expression did not show a significant correlation with adverse outcomes in terms of RFS and OS. Collectively, these findings suggest that increased GAPDH expression is specifically linked to reduced RFS and OS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD cancer types. Therefore, the next part of this study will focus on the unique role of GAPDH in these six types of human cancers.

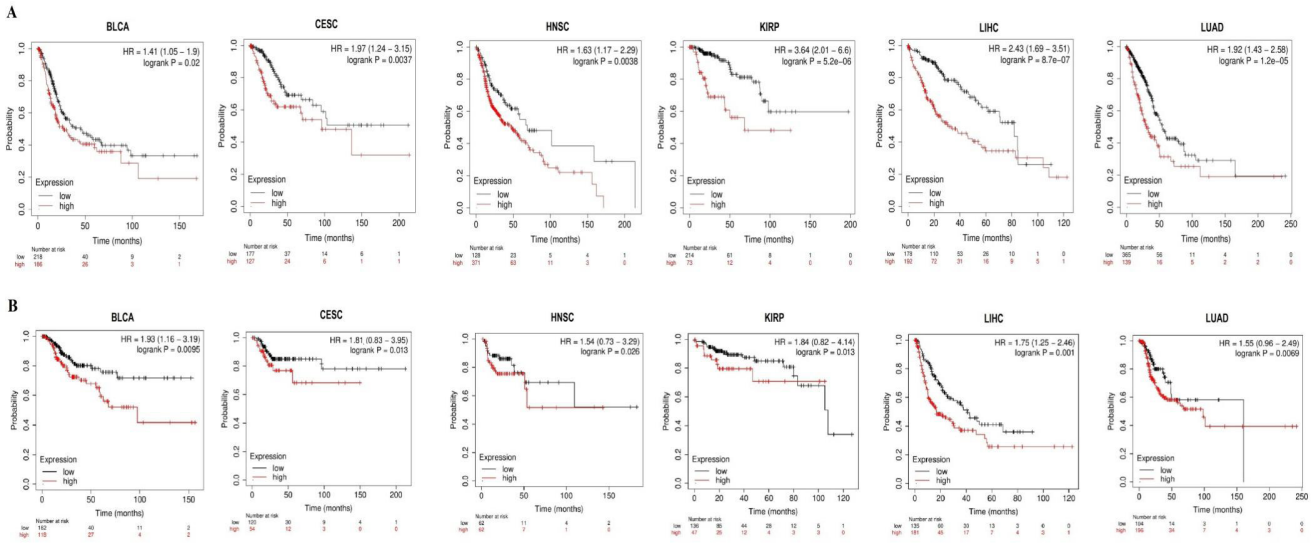

Figure 2. Elevated GAPDH linked to adverse RFS and OS in various cancer subtypes patients. **(A)** Association of GAPDH with RFS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. **(B)** Association of GAPDH with OS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. A *P*-value < 0.05 indicates significance.

3.3. Association between GAPDH expression and clinicopathological characteristics

An analysis of GAPDH expression across different clinicopathological features—including cancer stages, patient races, and nodal metastasis statuses—in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients revealed significant up-regulation of GAPDH ($P < 0.05$) relative to normal controls (**Figures 3–5**). The Student's *t*-test was used to compare GAPDH expression between groups, with a *P*-value < 0.05 indicating statistical significance.

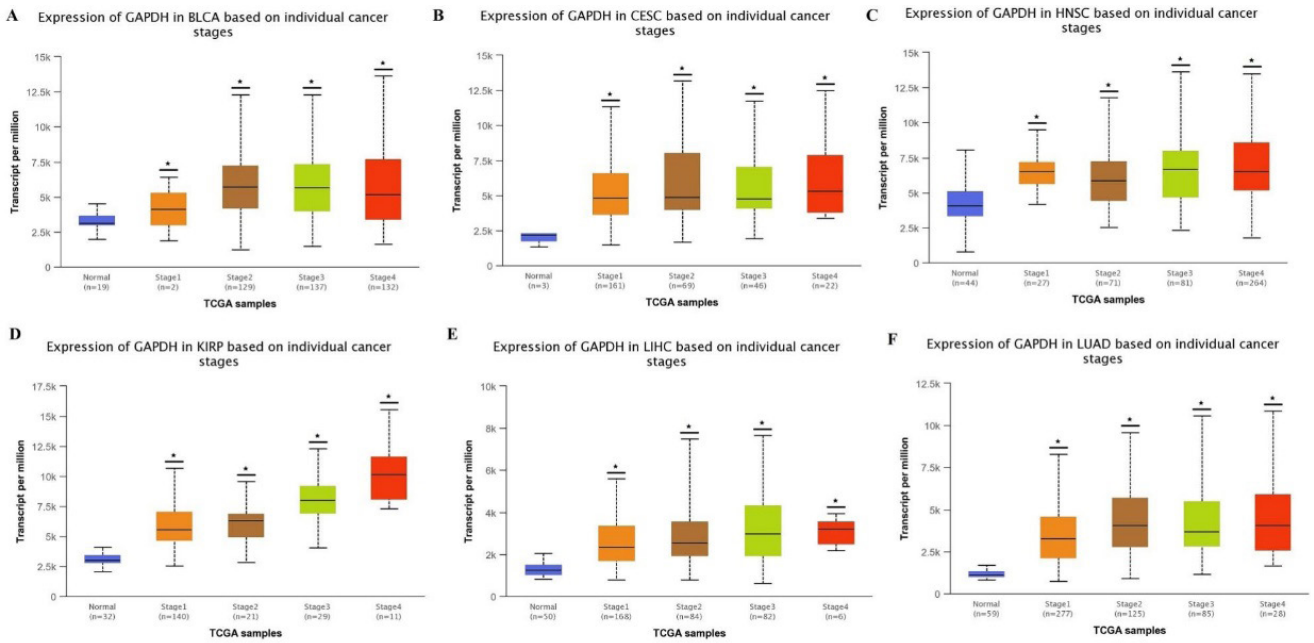

Figure 3. Cancer stage-specific expression patterns of GAPDH in various human cancers. **(A)** BLCA, **(B)** CESC, **(C)** HNSC, **(D)** KIRP, **(E)** LIHC, and **(F)** LUAD. A *P*-value < 0.05 was deemed statistically significant.

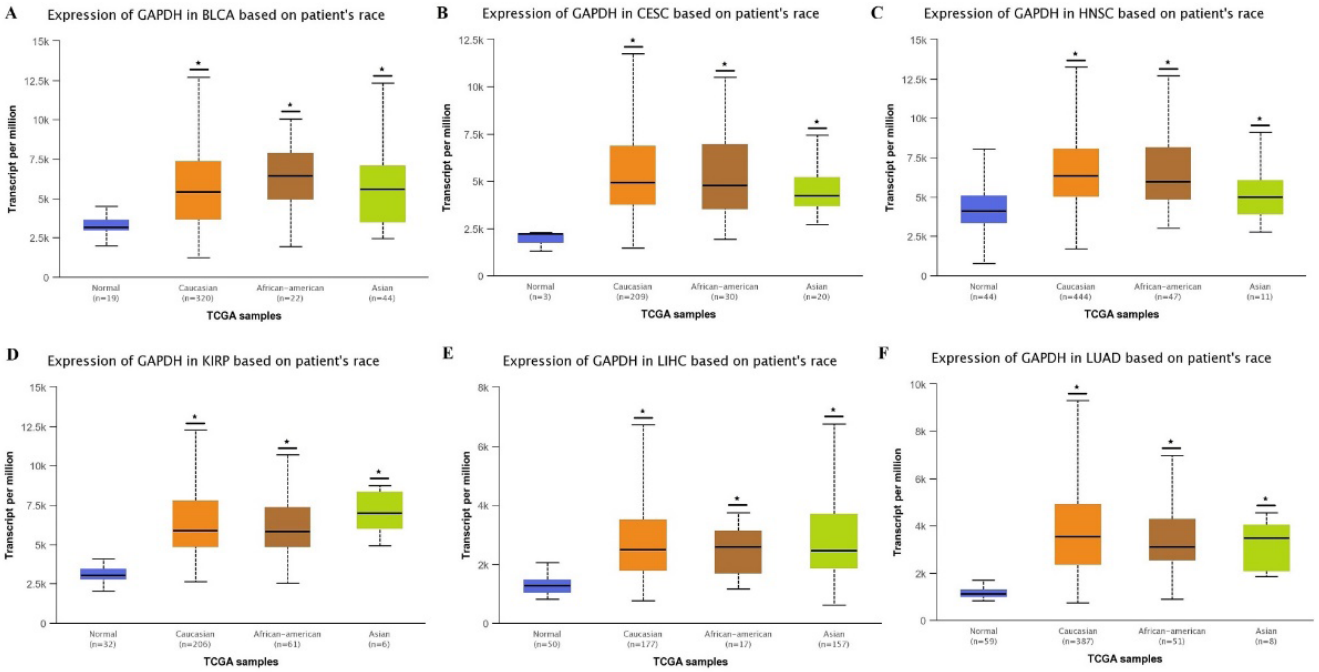

Figure 4. Race-specific expression patterns of GAPDH in various human cancers. **(A)** BLCA, **(B)** CESC, **(C)** HNSC, **(D)** KIRP, **(E)** LIHC, and **(F)** LUAD. A *P*-value < 0.05 was considered indicative of statistically significant results.

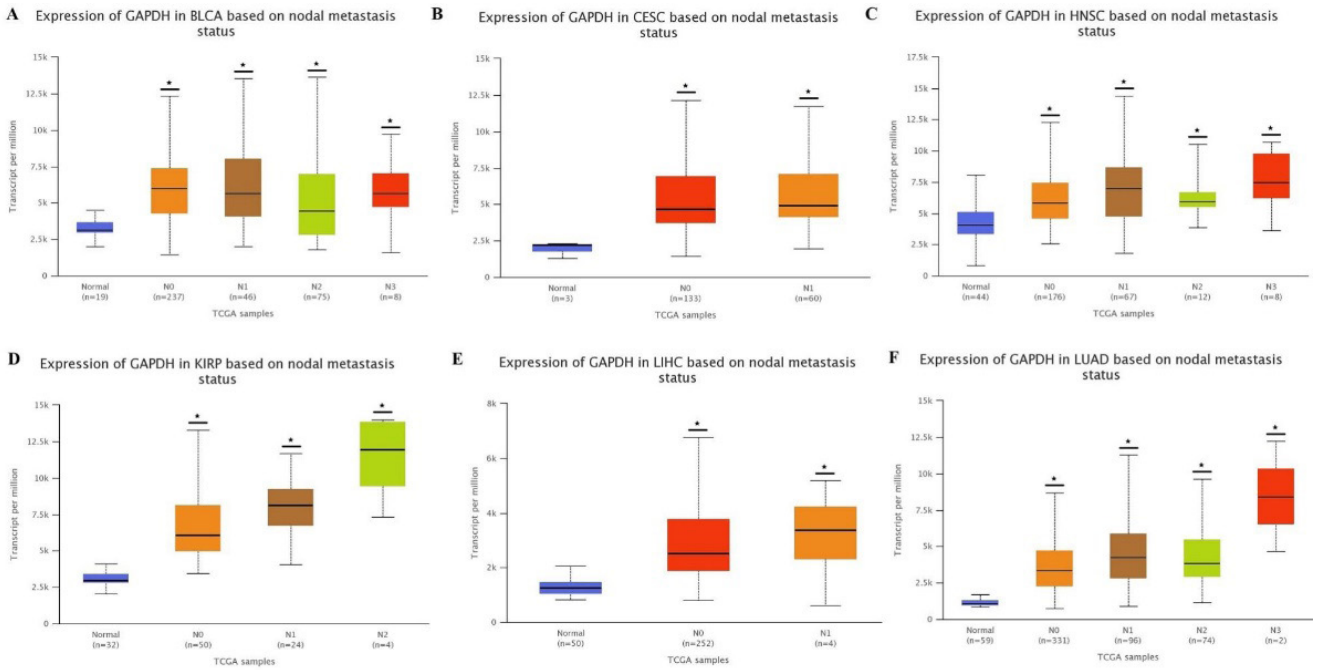

Figure 5. GAPDH expression profiles specific to nodal metastasis in various human cancers. **(A)** BLCA, **(B)** CESC, **(C)** HNSC, **(D)** KIRP, **(E)** LIHC, and **(F)** LUAD. A *P*-value < 0.05 was considered statistically significant.

Based on the observed trends in GAPDH expression across various cancer stages, it is evident that GAPDH expression is associated with distinct patterns in different cancer types. For instance, in BLCA, GAPDH expression significantly increases in stages 2 and 3, suggesting a potential role in disease progression during these stages. In CESC, GAPDH expression reaches its highest levels in stage 4, possibly indicating involvement in advanced disease states. Conversely, in HNSC, GAPDH expression is more pronounced in stage

1, possibly reflecting its role in early-stage cancer. In KIRP, GAPDH expression notably increases in stage 4, suggesting its significance in late-stage disease. In LIHC, GAPDH expression is most pronounced in stage 4, possibly indicating a role in advanced liver cancer. Finally, in LUAD, GAPDH expression is higher in stage 2, suggesting involvement in this particular stage of lung cancer. These findings underscore the complexity of GAPDH expression regulation across different cancer stages. The variations in GAPDH expression patterns may reflect its multifaceted roles in cellular processes and highlight its potential as a marker for specific stages of cancer.

Regarding GAPDH expression in patients of different racial backgrounds, the following patterns emerged: In BLCA patients, African-American individuals exhibited higher GAPDH expression levels compared to their Caucasian and Asian counterparts. Among CESC patients, GAPDH expression was elevated in Caucasian patients relative to African-American and Asian patients. Similarly, in HNSC patients, GAPDH expression was higher in Caucasian individuals compared to African-American and Asian patients. In KIRP patients, Asian individuals displayed higher GAPDH expression compared to African-American and Caucasian patients. In LIHC patients, African-American patients demonstrated higher GAPDH expression levels than Asian and Caucasian patients. Lastly, in LUAD patients, GAPDH expression was higher in Caucasian patients compared to Asian and African-American patients (**Figure 4**).

Concerning GAPDH expression in cancer patients with varying nodal metastasis statuses, distinct trends emerged across different cancer types: In BLCA patients, those with N0 status exhibited higher GAPDH expression levels compared to those with N1–N3 status. In CESC patients, those with N1 status displayed elevated GAPDH expression in comparison to those with N0 status. Among HNSC patients, those with N3 status demonstrated higher GAPDH expression levels than those with N0–N2 status. In KIRP patients, individuals with N2 status showed increased GAPDH expression relative to those with N0 and N1 statuses. In LIHC patients, those with N1 status exhibited higher GAPDH expression compared to those with N0 status. Finally, in LUAD patients, those with N4 status displayed elevated GAPDH expression compared to those with N0–N3 statuses (**Figure 5**).

Elevated GAPDH expression points to a metabolic shift known as the Warburg effect, commonly observed in cancer cells, where glycolysis is enhanced for rapid energy production and cell proliferation. This phenomenon not only signifies a potential diagnostic tool for early cancer detection but also underscores the aggressive nature of these cancers. Furthermore, these findings may pave the way for innovative therapeutic strategies targeting the glycolytic pathway, including GAPDH, to disrupt cancer cell metabolism and improve treatment outcomes.

3.4. Promoter methylation

The MEXPRESS database was utilized to examine the correlation between GAPDH promoter methylation and its expression in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. The analysis revealed a significant ($P < 0.05$) negative correlation between GAPDH promoter methylation levels and its expression in these cancer types (**Figure 6**).

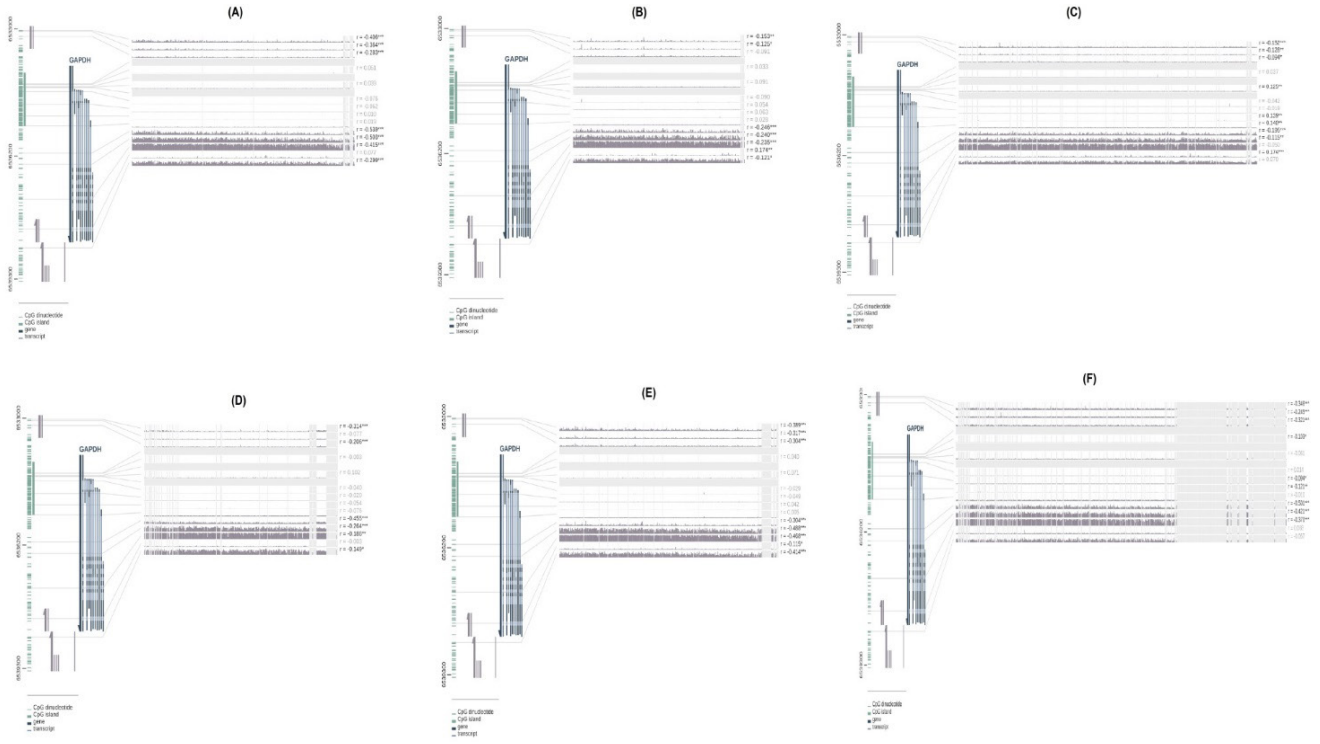

Figure 6. A correlation analysis between GAPDH expression and its promoter methylation level in different cancer subtypes via MEXPRESS. **(A)** In BLCA**, (B)** In CESC**, (C)** In HNSC, **(D)** In KIRP, **(E)** In LIHC, and **(F)** In LUAD**.** A *P*-value < 0.05 was considered to indicate the statistically significant results.

3.5. Genomic analysis

Genetic alterations in GAPDH within BLCA, CESC, HNSC, KIRP, LIHC, and LUAD were analyzed using various TCGA datasets, including "Bladder Urothelial Carcinoma (TCGA, Firehose Legacy, consisting of 413 cancerous samples), Cervical Squamous Cell Carcinoma (TCGA, PanCancer Atlas, consisting of 297 cancerous samples), Head and Neck Squamous Cell Carcinoma (TCGA, Firehose Legacy, consisting of 530 cancerous samples), Kidney Renal Clear Cell Carcinoma (TCGA, Firehose Legacy, consisting of 538 cancerous samples), Liver Hepatocellular Carcinoma (TCGA, Firehose Legacy, consisting of 379 cancerous samples), and Lung Adenocarcinoma (TCGA, Firehose Legacy, consisting of 586 cancerous samples)." The results revealed that GAPDH harbors genetic alterations in 2.4% of BLCA cases, with deep amplification being the most common alteration; 1.4% of CESC cases, with missense mutations being the most common; 2.4% of HNSC cases, with deep amplification; 0.5% of KIRP cases, with deep amplification; 0.4% of LIHC cases, with missense mutations; and 4% of LUAD cases, with deep deletions (**Figure 7A–F**). Moreover, GAPDH expression was assessed in two distinct groups: one comprising BLCA, CESC, HNSC, KIRP, LIHC, and LUAD samples without mutations in GAPDH, and the other consisting of samples from the same cancer types but with mutations in GAPDH. The results indicated no significant disparity in overall gene expression, including GAPDH expression, between these two groups (**Figure 7G**). These findings suggest that mutations in GAPDH do not influence its expression in the studied cancers.

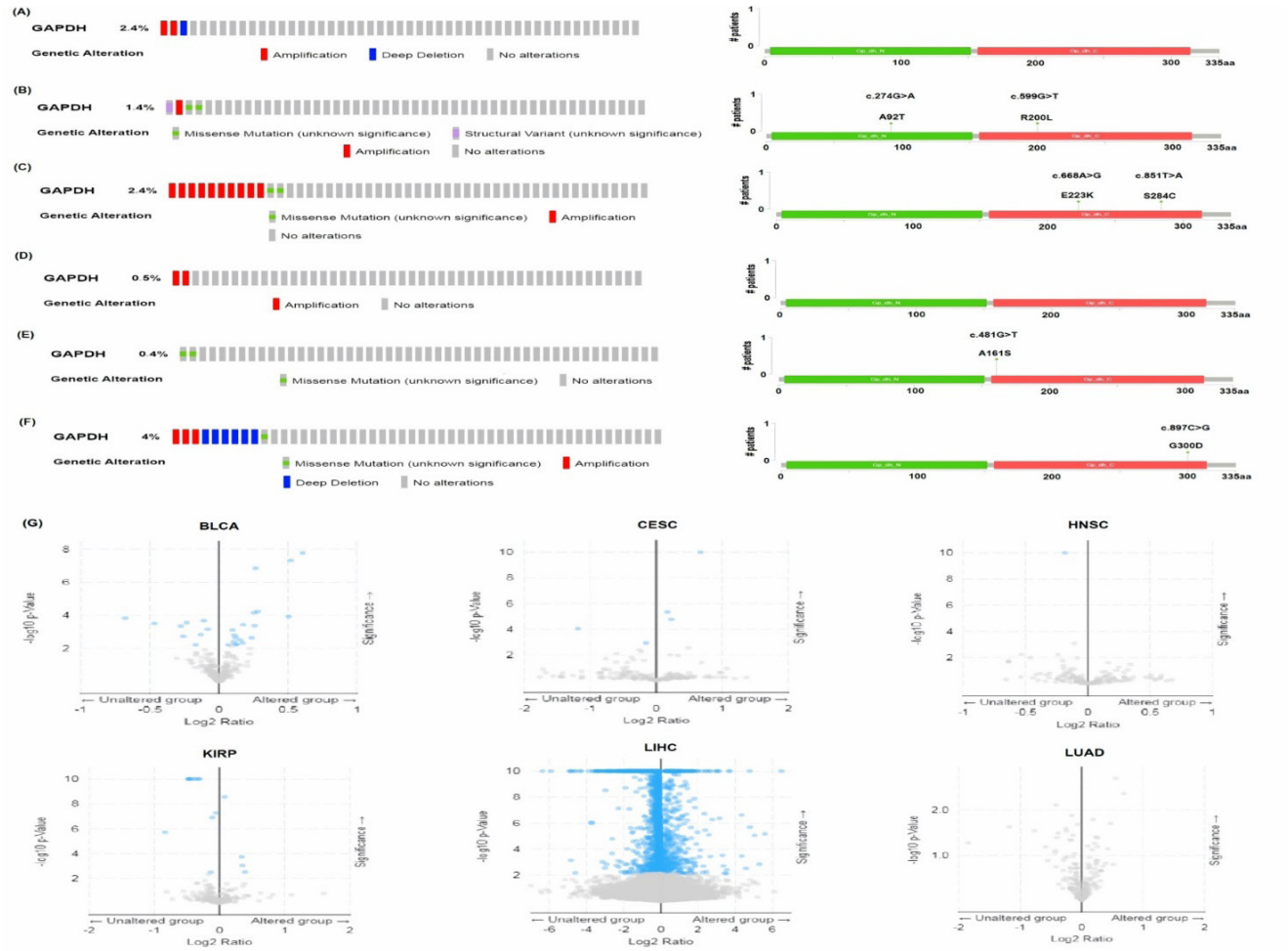

Figure 7. Analysis of genetic alterations in GAPDH across different TCGA datasets. **(A)** BLCA dataset, **(B)** CESC dataset, **(C)** HNSC dataset, **(D)** KIRP dataset, **(E)** LIHC dataset, and **(F)** LUAD dataset.

3.6. Co-expressed genes, PPI network, and pathway analysis

Firstly, GEPIA was used to identify GAPDH co-expressed genes in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. The analysis showed that TPI1, GAPDHP1, PHB2, ALDOA, YBX3, ENO1, PKM, MRPL51, USP5, and NCAPD2 are the top nine co-expressed genes with GAPDH (**Figure 8A**). These GAPDH-enriched genes were further processed for pathway analysis using the DAVID tool. The results revealed that GAPDH-enriched genes are significantly involved in various pathways, including "Fructose and Mannose Metabolism," "Glycolysis/Gluconeogenesis," "Biosynthesis of Amino Acids," and "Carbon Metabolism" (**Figure 8B**).

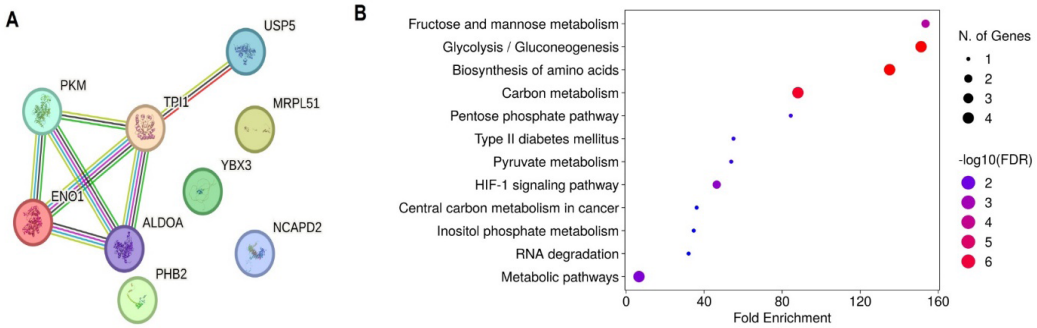

Figure 8. Construction of a protein-protein interaction (PPI) network and pathway analysis of genes enriched with GAPDH. **(A)** PPI network displaying the interactions among genes enriched with GAPDH, and **(B)** Pathway analysis illustrating the functional pathways associated with genes enriched with GAPDH.

3.7. GAPDH and infiltration level of CD8+ T cells

$CD8^+$ T immune cells play a crucial role in the success of current cancer immunotherapies [29]. Therefore, maintaining an appropriate level of $CD8^+$ T immune cells in cancer tissues is of utmost importance. In this study, TIMER was used to calculate the Spearman correlation between GAPDH expression and $CD8^+$ T cell levels. The results demonstrated a significant ($P < 0.05$) positive correlation between GAPDH expression and $CD8^+$ T cell levels in BLCA, while a significant ($P < 0.05$) negative correlation was observed in CESC, HNSC, KIRP, LIHC, and LUAD (**Figure 9**).

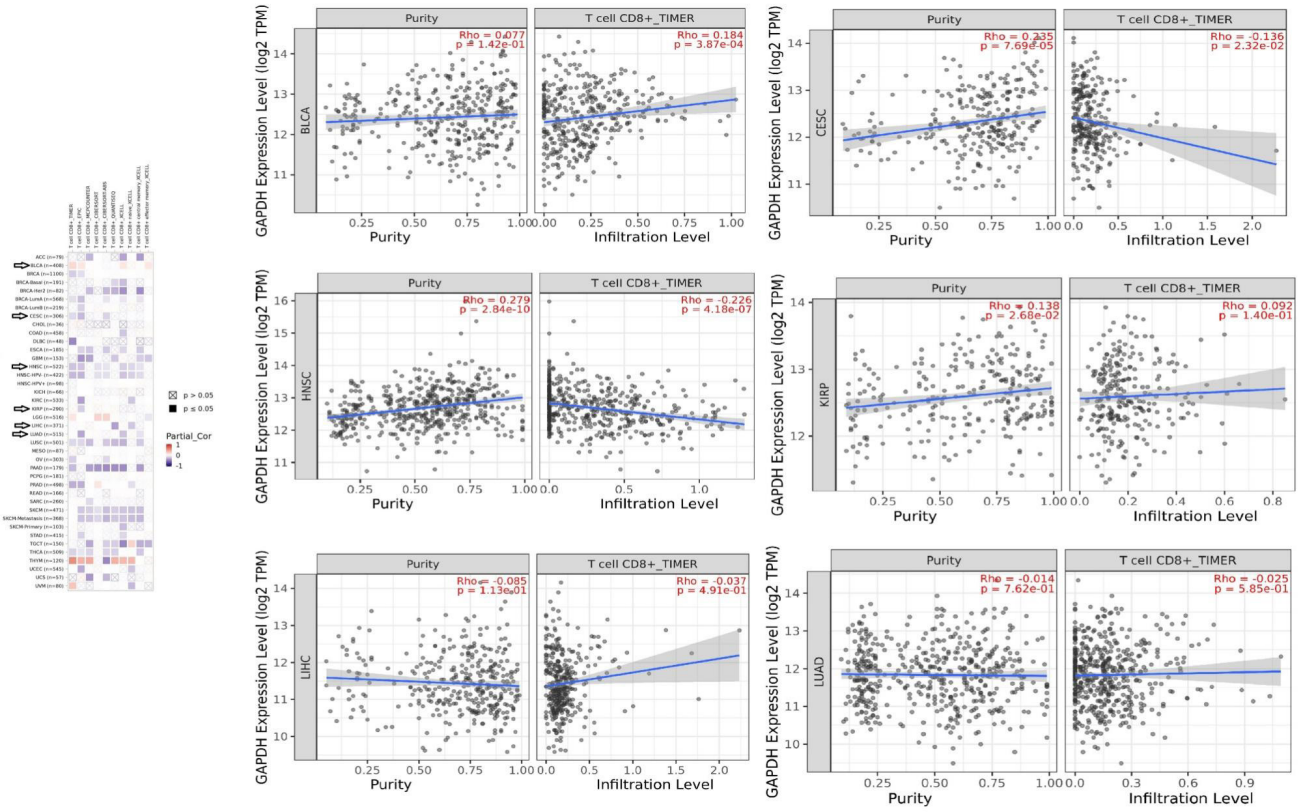

Figure 9. Correlational analysis of GAPDH expression and levels of $CD8^+$ T cells in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD using TIMER. A *P*-value < 0.05 was considered indicative of statistically significant results.

3.8. GAPDH-associated drugs

A gene-drug interaction network analysis was conducted to identify drugs associated with GAPDH using the CTD database. The analysis revealed several drugs that may regulate GAPDH expression. For instance, bisphenol A and tretinoin were found to increase GAPDH expression levels, while ethinyl estradiol and carbon tetrachloride were associated with a decrease in GAPDH expression levels (**Figure 10**). These findings suggest that these drugs may impact GAPDH expression and provide insights into potential therapeutic interventions.

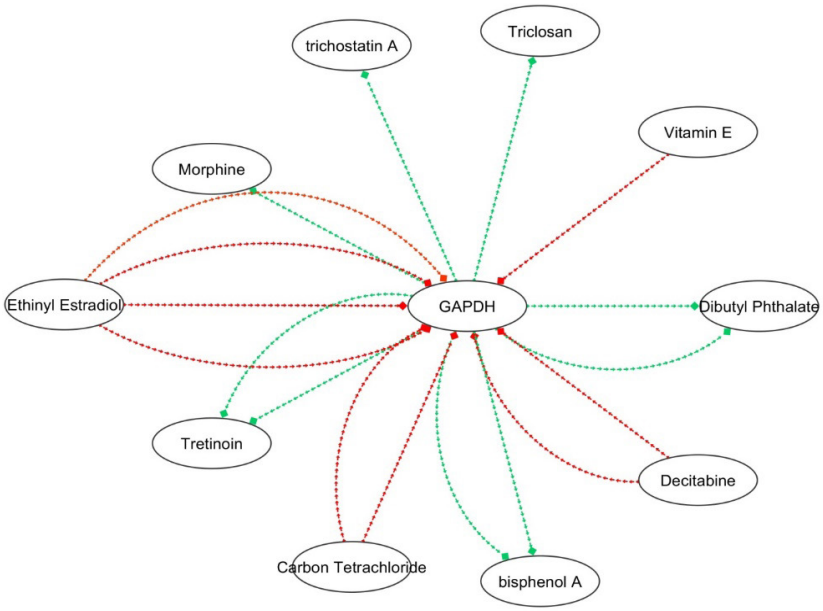

Figure 10. Network of drugs targeting GAPDH. The red color indicates drugs that can increase GAPDH expression, and the green arrows represent drugs that can decrease GAPDH expression. The numbers of arrows denote the reference count of selected drugs.

4. Discussion

Under normal physiological conditions, healthy cells primarily rely on oxidative phosphorylation, a more energy-efficient process, to generate adenosine triphosphate (ATP) [30]. However, in stark contrast, cancer cells often exhibit a metabolic shift known as the Warburg effect. This effect refers to the active utilization of glycolysis—the breakdown of glucose—for ATP production, even in the presence of sufficient oxygen. Despite the availability of oxygen, cancer cells demonstrate a preference for glycolysis, leading to increased glucose uptake and lactate production [30]. The Warburg effect is a hallmark of many cancer types and provides cancer cells with metabolic advantages, such as increased biosynthetic precursors and reduced dependence on oxygen for energy production. Understanding the mechanisms behind the Warburg effect is crucial for developing targeted therapeutic approaches that exploit the metabolic vulnerabilities of cancer cells while sparing normal cells [30].

GAPDH, a glycolytic enzyme, specifically catalyzes the conversion of glyceraldehyde-3-phosphate (G-3-P) to 1,3-diphosphoglycerate [31]. Additionally, GAPDH contributes to numerous other cellular functions, such as the export of nuclear tRNA, DNA repair, DNA replication, exocytosis, endocytosis, cytoskeletal organization, carcinogenesis, and cell death [32,33]. Although GAPDH is commonly used as an internal control, its expression variations have also been documented in various human cell lines [34]. Remarkably decreased expression of GAPDH has been observed in breast cancer, glioma, prostate cancer, liver cancer, colorectal cancer, pancreatic cancer, gastric cancer, melanoma, and bladder cancer [35]. Conversely, an increased level of GAPDH has been confirmed as a pro-apoptotic agent by Nakajima *et al.* [36]. Such variation in the expression of GAPDH across different cancer subtypes suggests its inconsistent role in determining cell fate [35].

To the best of our knowledge, no previous study has investigated the expression profile of GAPDH in different human cancer subtypes and its correlation with various clinicopathological features such as RFS, OS,

promoter methylation status, genetic alterations, CNVs, and $CD8^+$ T cell levels. Hence, this study aimed to examine the expression pattern of GAPDH across 24 types of human cancers and its association with diverse parameters, including RFS, OS, promoter methylation status, genetic alterations, CNVs, and $CD8^+$ T cell levels. By exploring these relationships, this study aimed to enhance the understanding of the potential role of GAPDH in cancer development and its significance as a biomarker in different cancer types.

GAPDH was observed to be upregulated in 24 major human cancers and its overexpression was significantly associated with decreased RFS and OS in patients with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. These findings suggest a crucial role for GAPDH in the development of these specific cancer subtypes. Therefore, this study focused primarily on BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. Furthermore, GAPDH was found to be significantly overexpressed ($P < 0.05$) in patients with these cancers across different clinicopathological features, including cancer stages, patient races, and nodal metastasis status, compared to normal controls. The increased expression of GAPDH throughout all stages of these cancers suggests its involvement not only in glycolysis-related processes but also in non-glycolytic mechanisms during tumor development. Moreover, the observed elevated expression of GAPDH in patients of different races with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD highlights the potential for race-independent treatment strategies in these patient populations. Additionally, the increased GAPDH expression in patients with different nodal metastasis statuses implies that GAPDH may also affect the prognosis of these patients.

To investigate the factors contributing to the overexpression of GAPDH in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD, a correlation analysis was performed using the MEXPRESS database to examine the relationship between GAPDH expression and its promoter methylation levels. The results revealed a significant negative correlation between GAPDH expression and promoter methylation levels, suggesting that hypomethylation may significantly influence GAPDH expression in these cancers. However, further experimental studies on a larger scale are necessary to validate and expand upon these findings.

Several biomarkers have been identified for the diagnosis and prognosis of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. For example, Brisuda *et al.* recently identified circulating tumor DNA (ctDNA) as a novel biomarker for BLCA [37]. Berger *et al.* identified various mutated genes as novel biomarkers for CESC by analyzing Gene GEO datasets [38]. Song *et al.* developed a long noncoding RNA-microRNA-mRNA network in CESC using GEO datasets, providing novel insights into CESC biology [39]. Sawyers *et al.* published a review article highlighting the role of novel HNSC molecular biomarkers, including EGFR, CCND1, Bcl-2, Kip1, VEGF, and p53 [40]. Similarly, various KIRP-related diagnostic and prognostic biomarkers have been identified, including VHL [41], VEGF [42], CAIX [43], and HIF-1**α**/2**α** [44]. Furthermore, the diagnostic and prognostic potential of different genes, including TTF-1, p63, CK5/6, napsin-A, SPATS2, and ST6GALNAC1, has been well-established in LUAD by previous studies [45]. However, there is a lack of generalization of any biomarkers in patients with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD with diverse clinicopathological features. In this study, we observed a significant upregulation of GAPDH expression in these patients compared to the control group. Additionally, our analysis of GAPDH promoter methylation levels, as well as the assessment of RFS and OS, supports its potential as a novel biomarker for BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients.

$CD8^+$ T cells play a pivotal role in the immune response against cancer [46]. This study revealed intriguing correlations between GAPDH expression and $CD8^+$ T cell levels in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. These findings suggest that GAPDH might modulate the immune response and contribute to the development of these cancers.

In this study, pathway analysis of GAPDH-enriched genes revealed their involvement in several KEGG pathways, including “Fructose and Mannose Metabolism,” “Glycolysis/Gluconeogenesis,” “Biosynthesis of

Amino Acids," and "Carbon Metabolism," among others. Additionally, a few potential drugs that could help prevent BLCA, CESC, HNSC, KIRP, LIHC, and LUAD were identified by controlling GAPDH expression.

5. Conclusion

In summary, this study has identified the diagnostic significance of GAPDH in patients with BLCA, CESC, HNSC, KIRP, LIHC, and LUAD across diverse clinicopathological features. The prognostic value of GAPDH was also assessed, thereby establishing correlations with its expression that could potentially aid in predicting the prognosis of patients with these cancers. However, further experimental investigations are warranted before translating these findings into clinical applications.

Disclosure statement

The author declares no conflict of interest.

References

[1] Brücher BL, Kitajima M, Siewert JR, 2014, Undervalued Criteria in the Evaluation of Multimodal Trials for Upper GI Cancers. Cancer Invest, 32(10): 497–506. https://doi.org/10.3109/07357907.2014.958497

[2] Feng Y, Spezia M, Huang S, et al., 2018, Breast Cancer Development and Progression: Risk Factors, Cancer Stem Cells, Signaling Pathways, Genomics, and Molecular Pathogenesis. Genes Dis, 5(2): 77–106. https://doi.org/10.1016/j.gendis.2018.05.001

[3] Stein KD, Syrjala KL, Andrykowski MA, 2008, Physical and Psychological Long-Term and Late Effects of Cancer. Cancer, 112(11 Suppl): 2577–2592. https://doi.org/10.1002/cncr.23448

[4] Anand P, Kunnumakkara AB, Sundaram C, et al., 2008, Cancer is A Preventable Disease That Requires Major Lifestyle Changes. Pharm Res, 25(9): 2097–2116. https://doi.org/10.1007/s11095-008-9661-9

[5] Lahtz C, Pfeifer GP, 2011, Epigenetic Changes of DNA Repair Genes in Cancer. J Mol Cell Biol, 3(1): 51–58. https://doi.org/10.1093/jmcb/mjq053

[6] Siegel RL, Miller KD, Jemal A, 2020, Cancer Statistics, 2020. CA Cancer J Clin, 70(1): 7–30. https://doi.org/10.3322/caac.21590

[7] Khurana E, Fu Y, Chakravarty D, et al., 2016, Role of Non-Coding Sequence Variants in Cancer. Nat Rev Genet, 17(2): 93–108. https://doi.org/10.1038/nrg.2015.17

[8] Sharma A, Jiang C, De S, 2018, Dissecting the Sources of Gene Expression Variation in A Pan-Cancer Analysis Identifies Novel Regulatory Mutations. Nucleic Acids Res, 46(9): 4370–4381. https://doi.org/10.1093/nar/gky271

[9] Lazarev VF, Guzhova IV, Margulis BA, 2020, Glyceraldehyde-3-Phosphate Dehydrogenase is a Multifaceted Therapeutic Target. Pharmaceutics, 12(5): 416. https://doi.org/10.3390/pharmaceutics12050416

[10] Mori R, Wang Q, Danenberg KD, et al., 2008, Both Beta-Actin and GAPDH are Useful Reference Genes for Normalization of Quantitative RT-PCR in Human FFPE Tissue Samples of Prostate Cancer. Prostate, 68(14): 1555–1560. https://doi.org/10.1002/pros.20815

[11] Murthi P, Fitzpatrick E, Borg AJ, et al., 2008, GAPDH, 18S rRNA and YWHAZ are Suitable Endogenous Reference Genes for Relative Gene Expression Studies in Placental Tissues from Human Idiopathic Fetal Growth Restriction. Placenta, 29(9): 798–801. https://doi.org/10.1016/j.placenta.2008.06.007

[12] Epner DE, Partin AW, Schalken JA, et al., 1993, Association of Glyceraldehyde-3-Phosphate Dehydrogenase Expression with Cell Motility and Metastatic Potential of Rat Prostatic Adenocarcinoma. Cancer Res, 53(9): 1995–1997.

[13] Tang Z, Yuan S, Hu Y, et al., 2012, Over-Expression of GAPDH in Human Colorectal Carcinoma as A Preferred Target of 3-Bromopyruvate Propyl Ester. J Bioenerg Biomembr, 44(1): 117–125. https://doi.org/10.1007/s10863-012-9420-9

[14] Seykora JT, Jih D, Elenitsas R, et al., 2003, Gene Expression Profiling of Melanocytic Lesions. Am J Dermatopathol, 25(1): 6–11. https://doi.org/10.1097/00000372-200302000-00002

[15] Giricz O, Lauer-Fields JL, Fields GB, 2008, The Normalization of Gene Expression Data in Melanoma: Investigating the Use of Glyceraldehyde 3-Phosphate Dehydrogenase and 18S Ribosomal RNA as Internal Reference Genes for Quantitative Real-Time PCR. Anal Biochem, 380(1): 137–139. https://doi.org/10.1016/j.ab.2008.05.024

[16] Zhou J, Dudley ME, Rosenberg SA, et al., 2005, Persistence of Multiple Tumor-Specific T-Cell Clones is Associated with Complete Tumor Regression in a Melanoma Patient Receiving Adoptive Cell Transfer Therapy. J Immunother, 28(1): 53–62. https://doi.org/10.1097/00002371-200501000-00007

[17] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al., 2017, UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia, 19(8): 649–658. https://doi.org/10.1016/j.neo.2017.05.002

[18] Maciejczyk A, Szelachowska J, Czapiga B, et al., 2013, Elevated BUBR1 Expression is Associated with Poor Survival in Early Breast Cancer Patients: 15-Year Follow-Up Analysis. J Histochem Cytochem, 61(5): 330–339. https://doi.org/10.1369/0022155413480148

[19] Koch A, De Meyer T, Jeschke J, et al., 2015, MEXPRESS: Visualizing Expression, DNA Methylation and Clinical TCGA Data. BMC Genomics, 16(1): 636. https://doi.org/10.1186/s12864-015-1847-z

[20] Cerami E, Gao J, Dogrusoz U, et al., 2012, The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discov, 2(5): 401–404. https://doi.org/10.1158/2159-8290.CD-12-0095

[21] Tang Z, Li C, Kang B, et al., 2017, GEPIA: A Web Server for Cancer and Normal Gene Expression Profiling and Interactive Analyses. Nucleic Acids Res, 45(W1): W98–W102. https://doi.org/10.1093/nar/gkx247

[22] von Mering C, Huynen M, Jaeggi D, et al., 2003, STRING: A Database of Predicted Functional Associations Between Proteins. Nucleic Acids Res, 31(1): 258–261. https://doi.org/10.1093/nar/gkg034

[23] Shannon P, Markiel A, Ozier O, et al., 2003, Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res, 13(11): 2498–2504. https://doi.org/10.1101/gr.1239303

[24] Huang DW, Sherman BT, Tan Q, et al., 2007, The DAVID Gene Functional Classification Tool: A Novel Biological Module-Centric Algorithm to Functionally Analyze Large Gene Lists. Genome Biol, 8(9): R183. https://doi.org/10.1186/gb-2007-8-9-r183

[25] Li T, Fu J, Zeng Z, et al., 2020, TIMER2.0 for Analysis of Tumor-Infiltrating Immune Cells. Nucleic Acids Res, 48(W1): W509–W514. https://doi.org/10.1093/nar/gkaa407

[26] Mattingly CJ, Colby GT, Forrest JN, et al., 2003, The Comparative Toxicogenomics Database (CTD). Environ Health Perspect, 111(6): 793–795. https://doi.org/10.1289/ehp.6028

[27] Rio DC, Ares M Jr, Hannon GJ, et al., 2010, Purification of RNA Using TRIzol (TRI Reagent). Cold Spring Harb Protoc, 2010(6): pdb.prot5439. https://doi.org/10.1101/pdb.prot5439

[28] Ghatak S, Muthukumaran RB, Nachimuthu SK, 2013, A Simple Method of Genomic DNA Extraction From Human Samples for PCR-RFLP Analysis. J Biomol Tech, 24(4): 224–231. https://doi.org/10.7171/jbt.13-2404-001

[29] Ziai J, Gilbert HN, Foreman O, et al., 2018, $CD8^+$ T Cell Infiltration in Breast and Colon Cancer: A histologic and Statistical Analysis. PLoS One, 13(1): e0190158. https://doi.org/10.1371/journal.pone.0190158

[30] Warburg O, 1956, On the Origin of Cancer Cells. Science, 123(3191): 309–314. https://doi.org/10.1126/science.123.3191.309

[31] Wang J, Li Y, Pan L, et al., 2021, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Moonlights as An Adhesin in *Mycoplasma hyorhinis* Adhesion to Epithelial Cells as well as A Plasminogen Receptor Mediating Extracellular Matrix

Degradation. Vet Res, 52(1): 80. https://doi.org/10.1186/s13567-021-00952-8

[32] Colell A, Green DR, Ricci JE, 2009, Novel Roles for GAPDH in Cell Death and Carcinogenesis. Cell Death Differ, 16(12): 1573–1581. https://doi.org/10.1038/cdd.2009.137

[33] Ganapathy-Kanniappan S, Kunjithapatham R, Geschwind JF, 2012, Glyceraldehyde-3-Phosphate Dehydrogenase: A Promising Target for Molecular Therapy in Hepatocellular Carcinoma. Oncotarget, 3(9): 940–953. https://doi.org/10.18632/oncotarget.623

[34] Caradec J, Sirab N, Revaud D, et al., 2010, Is GAPDH A Relevant Housekeeping Gene for Normalisation in Colorectal Cancer Experiments? Br J Cancer, 103(9): 1475–1476. https://doi.org/10.1038/sj.bjc.6605851

[35] Guo C, Liu S, Sun MZ, 2013, Novel Insight Into The Role of GAPDH Playing in Tumor. Clin Transl Oncol, 15(3): 167–172. https://doi.org/10.1007/s12094-012-0924-x

[36] Nakajima H, Amano W, Fujita A, et al., 2007, The Active Site Cysteine of the Proapoptotic Protein Glyceraldehyde-3-Phosphate Dehydrogenase is Essential in Oxidative Stress-Induced Aggregation and Cell Death. J Biol Chem, 282(36): 26562–26574. https://doi.org/10.1074/jbc.M704199200

[37] Brisuda A, Pazourkova E, Soukup V, et al., 2016, Urinary Cell-Free DNA Quantification as Non-Invasive Biomarker in Patients with Bladder Cancer. Urol Int, 96(1): 25–31. https://doi.org/10.1159/000438828

[38] Berger AC, Korkut A, Kanchi RS, et al., 2018, A Comprehensive Pan-Cancer Molecular Study of Gynecologic and Breast Cancers. Cancer Cell, 33(4): 690–705.e9. https://doi.org/10.1016/j.ccell.2018.03.014

[39] Song J, Ye A, Jiang E, et al., 2018, Reconstruction and Analysis of the Aberrant lncRNA-miRNA-mRNA Network Based on Competitive Endogenous RNA in CESC. J Cell Biochem, 119(8): 6665–6673. https://doi.org/10.1002/jcb.26850

[40] Sawyers CL, 2008, The Cancer Biomarker Problem. Nature, 452(7187): 548–552. https://doi.org/10.1038/nature06913

[41] Labrousse-Arias D, Martínez-Alonso E, Corral-Escariz M, et al., 2017, VHL Promotes Immune Response Against Renal Cell Carcinoma via NF-κB-Dependent Regulation of VCAM-1. J Cell Biol, 216(3): 835–847. https://doi.org/10.1083/jcb.201608024

[42] Hou Q, Li MY, Huang WT, et al., 2017, Association Between Three VEGF Polymorphisms and Renal Cell Carcinoma Susceptibility: A Meta-Analysis. Oncotarget, 8(30): 50061–50070. https://doi.org/10.18632/oncotarget.17833

[43] Yang X, Minn I, Rowe SP, et al., 2015, Imaging of Carbonic Anhydrase IX with An ^{111}In-labeled Dual-motif Inhibitor. Oncotarget, 6: 33733–33742. https://doi.org/10.18632/oncotarget.5254

[44] Lai XM, Liu SY, Tsai YT, et al., 2017, HAF Mediates the Evasive Resistance of Anti-angiogenesis TKI Through Disrupting HIF-1α and HIF-2α Balance in Renal Cell Carcinoma. Oncotarget, 8(30): 49713–49724. https://doi.org/10.18632/oncotarget.17923

[45] Alabiad MA, Harb OA, Abozaid M, et al., 2021, The Diagnostic and Prognostic Roles of Combined Expression of Novel Biomarkers in Lung Adenocarcinoma and Lung Squamous Cell Carcinoma: An Immunohistochemical Study. Iran J Pathol, 16(2): 162–173. https://doi.org/10.30699/IJP.2020.130944.2452

[46] van der Leun AM, Thommen DS, Schumacher TN, 2020, $CD8^+$ T Cell States in Human Cancer: Insights from Single-Cell Analysis. Nat Rev Cancer, 20(4): 218–232. https://doi.org/10.1038/s41568-019-0235-4