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

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Abstract: *Background:* The present study aimed to investigate the expression level 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 that involved multiple layers of analysis. *Methods:* GAPDH expression and methylation levels were assessed via bioinformatics tools and validated using cell lines through RNA sequencing and targeted bisulfite sequencing analyses. The potential prognostic significance of GAPDH was evaluated through the use of a Kaplan–Meier 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. Lastly, a gene-drug interaction network analysis was conducted using Cytoscape to examine the relationship between GAPDH and drugs. *Results:* The GAPDH was found commonly up-regulated in 24 types of human cancers and its up-regulation was significantly correlated with the poor relapse-free survival (RFS) and overall survival (OS) of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. This implies that GAPDH plays a significant role in the development of these cancers. The GAPDH up-regulation was also noticed to be associated with the different clinicopathological features of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients. Pathway analysis has shown GAPDH involvement in different diverse pathways. Furthermore, notable correlations were observed between the expression of GAPDH and its promoter methylation level, genetic alterations, as well as the level of $CD8^+$ T immune cells. Moreover, we identified significant regulatory drugs targeting GAPDH that have 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 emerged 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 particular disease but a collection of various diseases [1]. It is characterized by the uncontrolled growth and spread of abnormal cells in the body. Cancer can arise in any part of the body and can 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, as it primarily results from variations in the expressions of various DNA repair and tumor suppressor genes [5]. In line with 2019 disease prevalence and mortality statistics, cancer has been declared as the second leading cause of death worldwide with an estimated 9.6 million deaths, or one in six deaths after cardiovascular diseases [6].

Recently, an increasing body of evidence 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]. Identification of the regulatory alterations and their effect on the gene expression level is an important aspect of understanding cancer biology. Mainly, the major cancer subtypes including breast cancer, colorectal cancer, and leukemia have usually 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 is yet 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 blot and reverse transcription-polymerase chain reaction [10,11]. Earlier, the GAPDH up-regulation appears to be linked with cancer development, as the initial evidence was found in the Dunning R-3327 rat prostatic adenocarcinoma, where a higher expression of GAPDH was documented in cancer cells as compared to the ventral prostate tissue of normal rat [12]. In addition, another study by Tang *et al.* [13] has also reported higher GAPDH expression in human colon cancer (CC) tissue as compared to the controls, which was further found elevated in metastatic liver tissue, suggesting that GAPDH may contribute to colon cancer metastasis. In contrast, Seykora *et al.* have 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 a significant up-regulation of GAPDH in melanoma tissues as compared to normal tissues [15]. Furthermore, recent studies have revealed the identification of point mutations in the GAPDH gene as a novel melanoma tumor antigen. These mutations have been found to be recognized by tumor-infiltrating T-lymphocytes in a patient with metastatic melanoma [16].

However, the biomarker role of GAPDH in various other human cancers is less 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 had not been conducted before. By performing this pan-cancer analysis, our study brings to light 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 the data of expression, methylation,

and clinicopathological parameters-based data [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 access to processed and normalized transcriptome sequencing data for multiple cancer types, including lung, breast, colorectal, ovarian, and other types of cancer. One of the most significant benefits of the UALCAN database is that it aims to bridge the gap between cancer genomics data and easy-to-use visualization and statistical computation tools. The platform provides researchers with the option to explore cancer transcriptomic data easily and intuitively. The platform incorporates several analytical tools, including gene expression analysis, patient survival analysis, tumor mutation burden analysis, and more [17]. The gene expression module is one of the essential UALCAN features. It enables researchers to analyze the gene expression levels of their genes of interest. They can also compare the expression levels of their genes of interest with normal tissue samples and among tumor subgroups. Moreover, the gene expression tool provides charts, gene expression box plots, and heat maps. For statistics purposes, a student t-test was used by UALCAN. A *P* value of < 0.05 was considered statistically significant results.

2.2. Kaplan–Meier plotter

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

2.3. MEXPRESS

MEXPRESS [19] was assessed in our 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 subtypes of cancer. Furthermore, users can 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 trustworthiness that helps researchers gain insights into cancer biology in a straightforward manner [19]. A *P* value of < 0.05 was deemed significant.

2.4. cBioPortal

In our study, genetic alterations and copy number variation (CNVs) in GAPDH as well as their correlation with the expression level of GAPDH 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, mutation, copy number variation, protein expression, and clinical information for thousands of cancer patients across numerous cancer types. Researchers can also find

and investigate potential cancer drivers, mutations, and clinical associations using a range of algorithms and visualizations [20].

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

GEPIA [21] database was used to identify co-express genes with GAPDH. STRING database [22] was used to construct a PPI network of the GAPDH genes via STRING using default settings. Subsequently, the PPI network was visualized using Cytoscape software 3.8.2 [23]. Furthermore, pathway analysis of the GAPDH-enriched genes was carried out using an online tool DAVID [24]. This tool 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, such as 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. The DAVID tool bridges the gap between the raw data and biological understanding by providing a comprehensive analysis of gene expression data. A P value of < 0.05 was considered significant.

2.6. GAPDH and infiltrating level of $CD8^+$ T cells

The Spearman correlation between GAPDH expression and $CD8^+$ T immune markers in BLCA (bladder urothelial carcinoma), CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), HNSC (head and neck squamous cell carcinoma), KIRP (kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), and LUAD (lung adenocarcinoma) patients was performed through a user-friendly resource, TIMER [25]. TIMER database includes 108 cancer types from the TCGA project. The 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. The database uses a deconvolution algorithm that integrates multiple immune cell-specific markers to estimate the abundance of immune cells [25]. A P value of < 0.05 was considered significant.

2.7. Screening of GAPDH regulatory drugs

CTD database [26] is used in this study to find the GAPDH regulatory drug including both positive and negative regulated drugs. CTD is a valuable resource in the field 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. Later, the identified drugs were visualized via Cytoscape 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 [27]. The results revealed a statistically significant ($P < 0.05$) overexpression of GAPDH in various human cancer samples, including BLCA, CESC, HNSC, KIRP, LIHC, and LUAD when compared to normal controls (**Figure 1**).

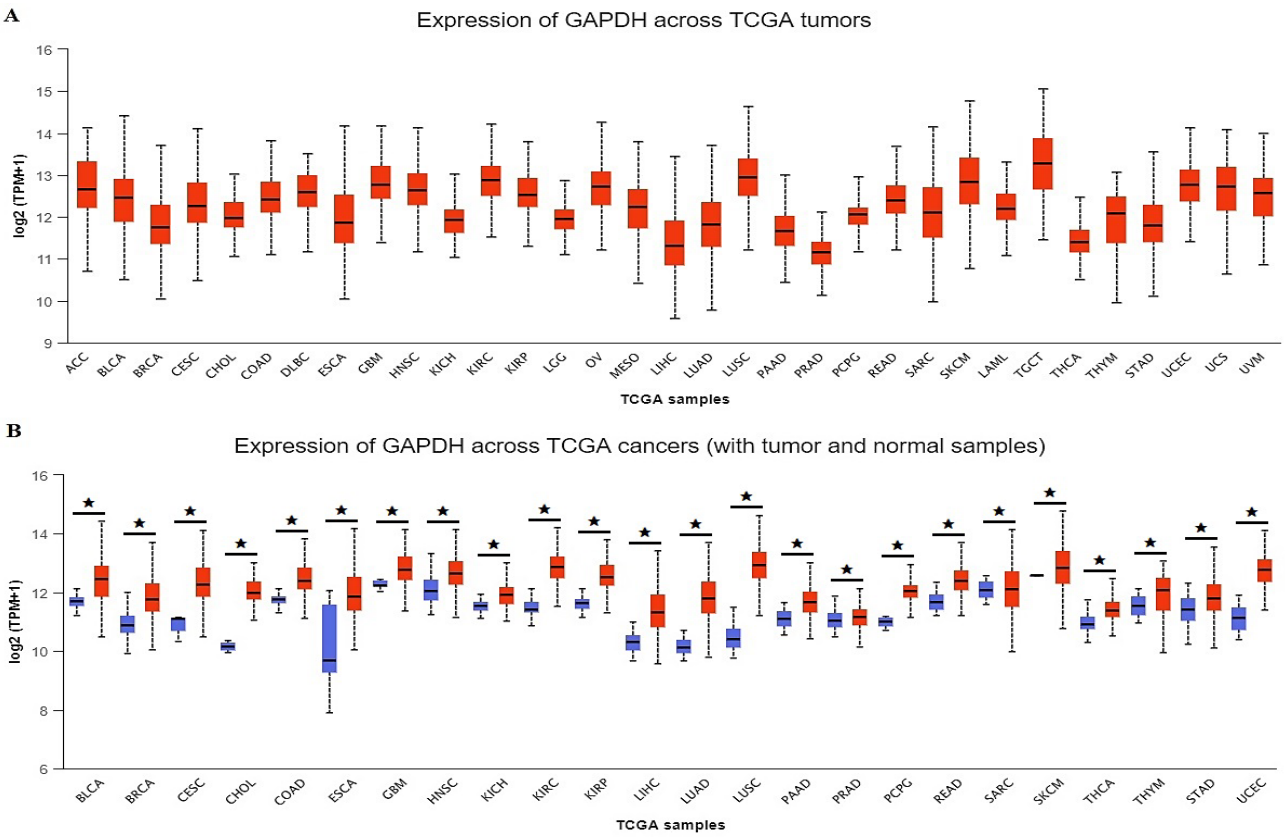

Figure 1. Profile of GAPDH expression across 24 different human cancers. (A) Expression profile of GAPDH in cancer samples exclusively. (B) Expression profile of GAPDH in both cancer samples and normal controls. $P < 0.05$ indicates significance.

3.2. GAPDH prognostic potential

Prognostic analysis of GAPDH in 24 cancer types was carried out using the KM plotter online tool. This analysis aimed to assess the relationship between GAPDH expression and the prognosis in terms of RFS and OS in 24 cancer types. It was observed that overexpressed GAPDH was significantly ($P < 0.05$) linked with the decreased RFS and OS duration of the 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, $P = 5.2e\text{-}06$; HR = 1.84, 95% CI: 0.82–4.14, $P = 0.013$), LIHC (HR = 2.43, 95% CI: 1.69–3.51, $P = 8.7e\text{-}07$; HR = 1.75, 95% CI: 1.25–2.46, $P = 0.001$), and LUAD (HR = 1.92, 95% CI: 1.43–2.58, $P = 1.2e\text{-}05$; HR = 1.55, 95% CI: 0.96–2.49, $P = 0.0069$) patients (**Figure 2**). Nevertheless, in the context of other cancer types, elevated GPDH expression did not demonstrate 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 our study will mainly focus on the unique role of GAPDH in these six types of human cancers.

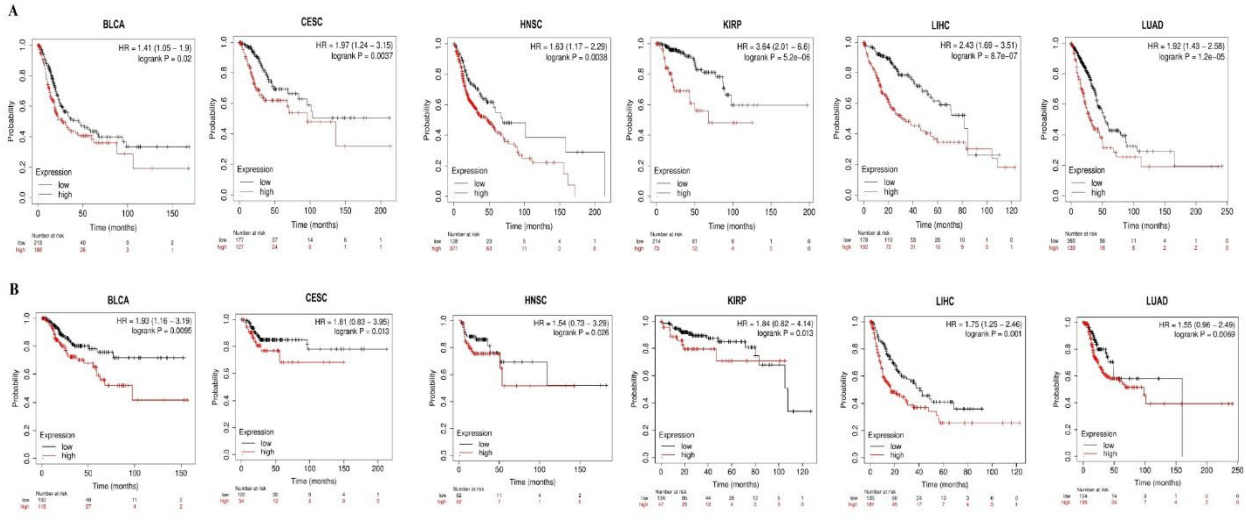

Figure 2. Elevated GAPDH linked to adverse RFS and OS in various cancer subtypes patients. (A) Association of GAPDH with RFS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. (B) Association of GAPDH with OS in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. $P < 0.05$ indicates significance.

3.3. Association between GAPDH expression and clinicopathological characteristics

Expression analysis of GAPDH expression across different clinicopathological features including different cancer stages, patient's races, and nodal metastasis statuses of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients has shown significant ($P < 0.05$) up-regulation of GAPDH relative to normal controls (**Figures 3** to **5**). The Student's *t*-test was employed for comparing the GAPDH expression. This test evaluated whether the means of the two groups were statistically different from each other, with $P < 0.05$ indicating the level of statistical significance.

Based on the observed trends in GAPDH expression across various cancer stages, it becomes evident that the expression of GAPDH is associated with distinct patterns in different types of cancer. In BLCA, for instance, GAPDH expression tends to rise significantly 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 its involvement in advanced disease states. Conversely, in HNSC, GAPDH expression is more pronounced in stage 1, possibly reflecting its role in early-stage cancer. For KIRP, GAPDH expression appears to increase notably in stage 4, suggesting its potential significance in late-stage disease. In LIHC, GAPDH expression is more pronounced in stage 4, possibly indicating a role in advanced liver cancer. Finally, for LUAD, GAPDH expression is higher in stage 2, suggesting its involvement in this particular stage of lung cancer. These findings underscore the complexity of GAPDH expression regulation in 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 also 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, enhancing glycolysis for rapid energy production and cell proliferation [28]. 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.

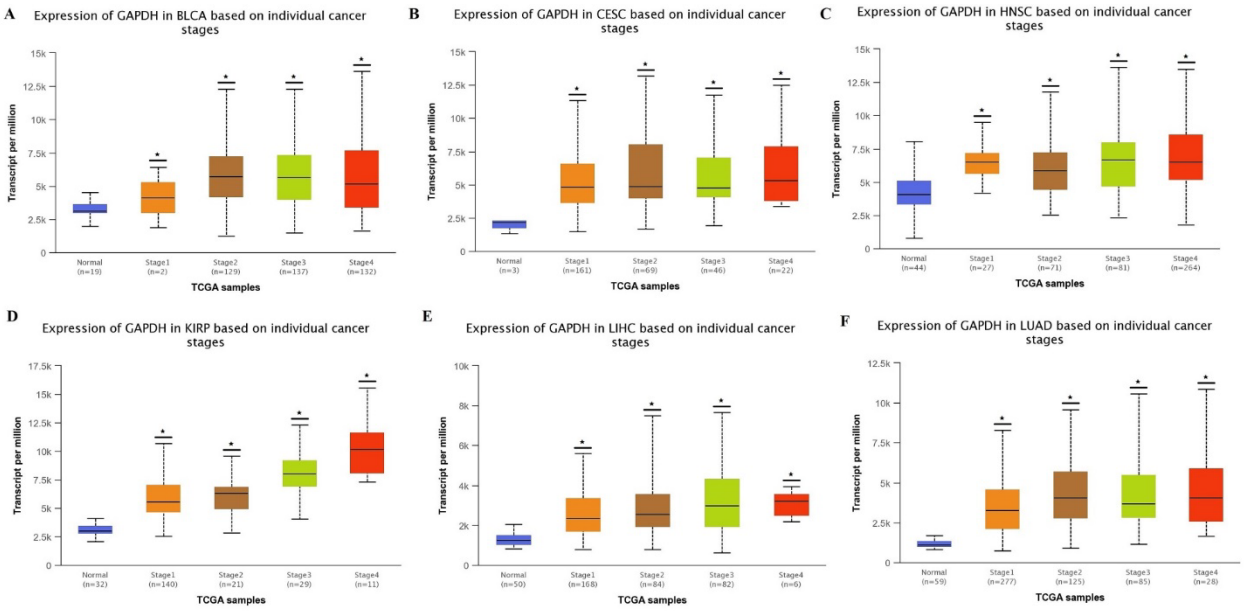

Figure 3. Cancer stage-specific expression patterns of GAPDH in various human cancers. (A) BLCA, (B) CESC, (C) HNSC, (D) KIRP, (E) LIHC, and (F) LUAD. $P < 0.05$ was deemed statistically significant.

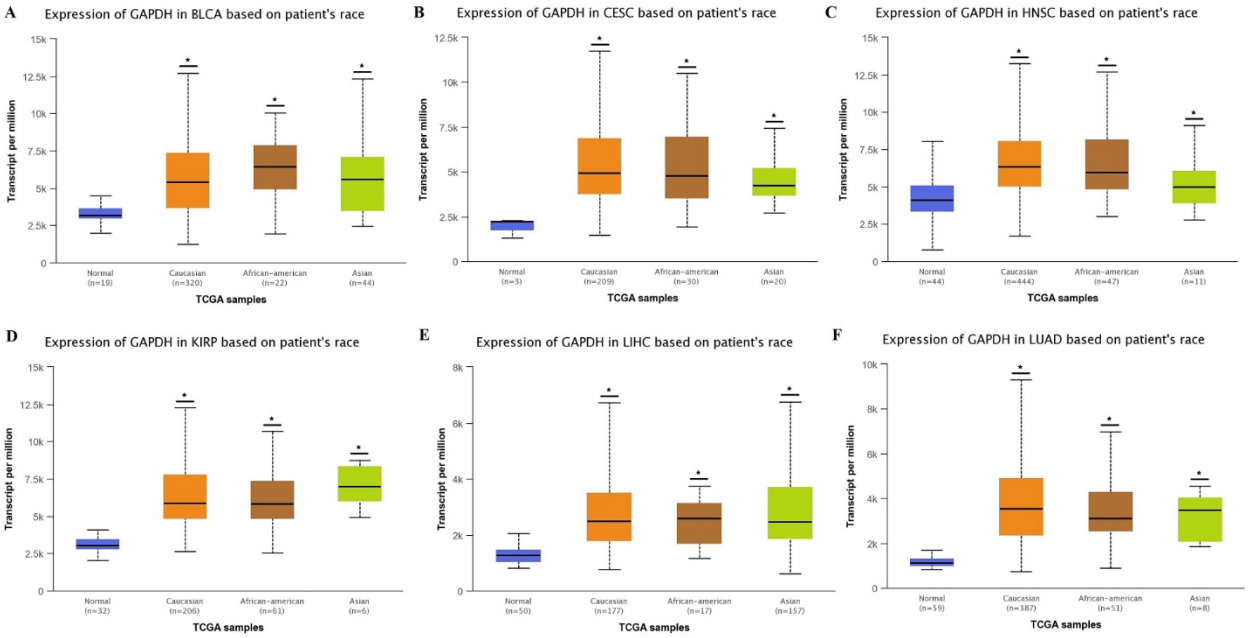

Figure 4. Race-specific expression patterns of GAPDH in various human cancers. (A) BLCA, (B) CESC, (C) HNSC, (D) KIRP, (E) LIHC, and (F) LUAD. $P < 0.05$ was considered indicative of statistically significant results.

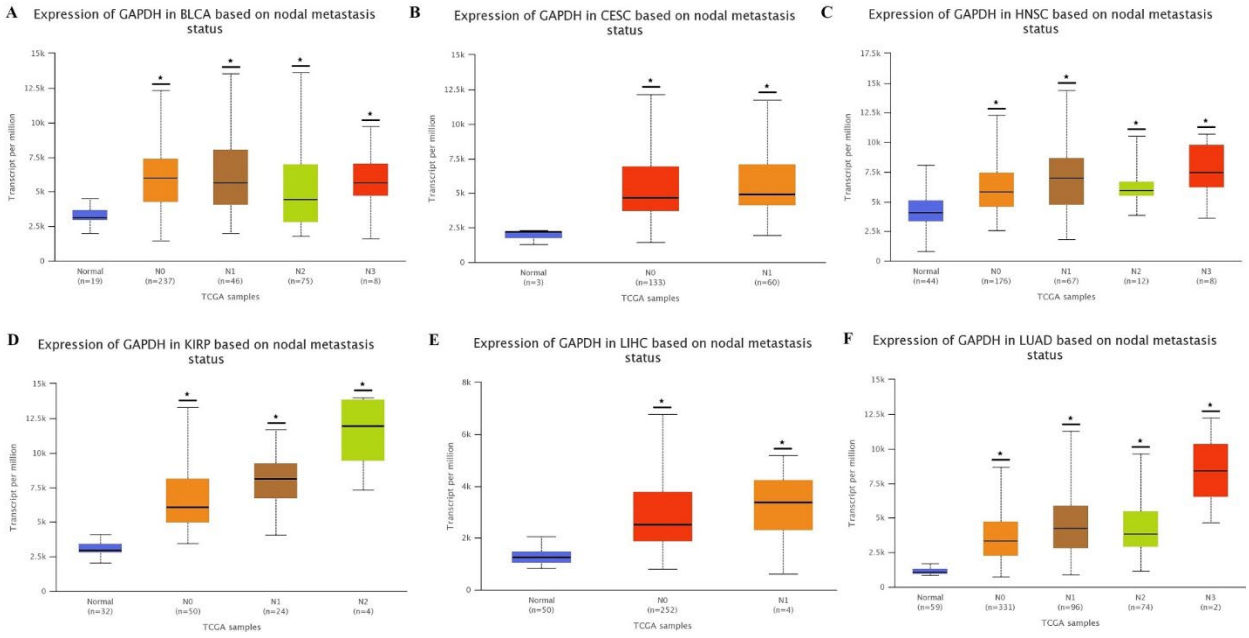

Figure 5. GAPDH expression profiles specific to nodal metastasis in various human cancers. (A) BLCA, (B) CESC, (C) HNSC, (D) KIRP, (E) LIHC, and (F) LUAD. $P < 0.05$ was considered statistically significant.

3.4. Promoter methylation

The MEXPRESS database was employed 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 level and its expression in these cancer types (**Figure 6**).

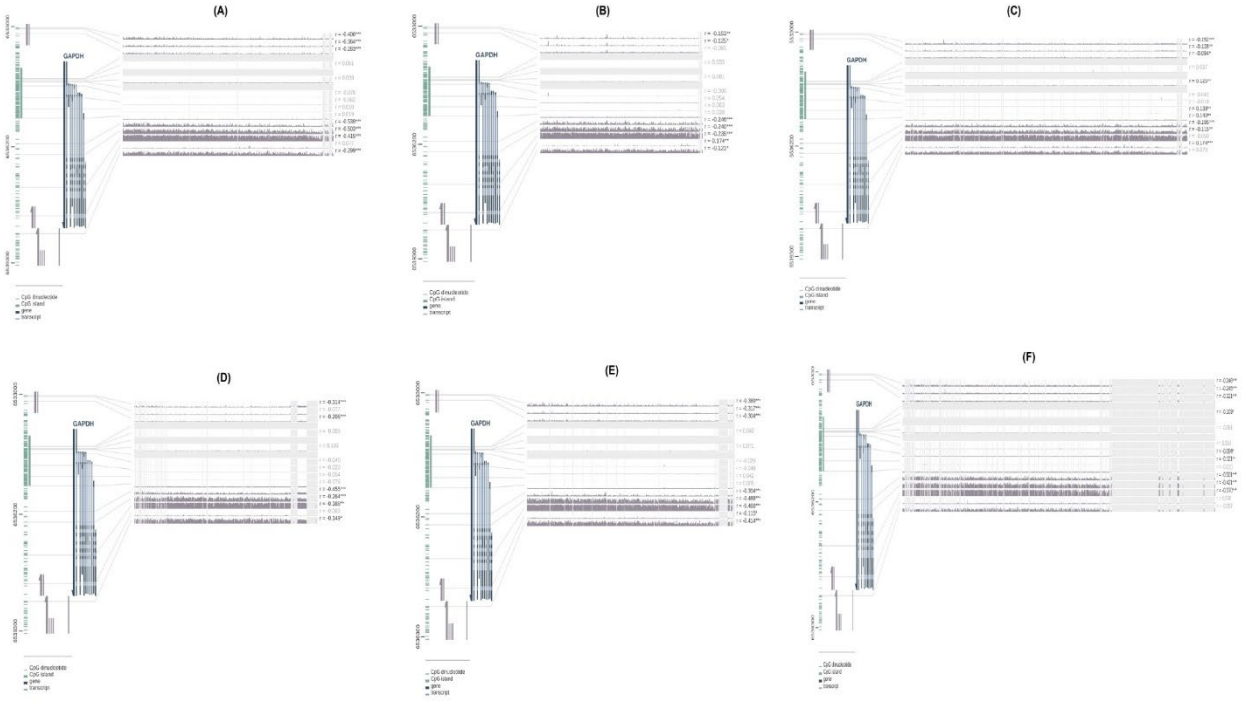

Figure 6. A correlation analysis between GAPDH expression and its promoter methylation level in different cancer subtypes via MEXPRESS. (A) In BLCA, (B) In CESC, (C) In HNSC, (D) In KIRP, (E) In LIHC, and (F) In LUAD. $P < 0.05$ was considered to indicate statistically significant results.

3.5. Genomic analysis

Details regarding genetic alterations in GAPDH within BLCA, CESC, HNSC, KIRP, LIHC, and LUAD were extracted from 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)." Results revealed that GAPDH harbors genetic alterations in 2.4% cases of the BLCA with maximum deep amplification, 1.4% cases of the CESC patients with maximum missense mutations, 2.4% cases of the HNSC patients with deep amplification, 0.5% cases of the KIRP with deep amplification, and 0.4% cases of the LIHC with missense mutations, and 4% cases of the LUAD patients with maximum deep deletion (**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 that there was no significant disparity in overall gene expression, including the expression of GAPDH between these two sample groups (**Figure 7G**). These findings suggest that mutations in GAPDH have no involvement in the regulation of its expression across the studied cancers.

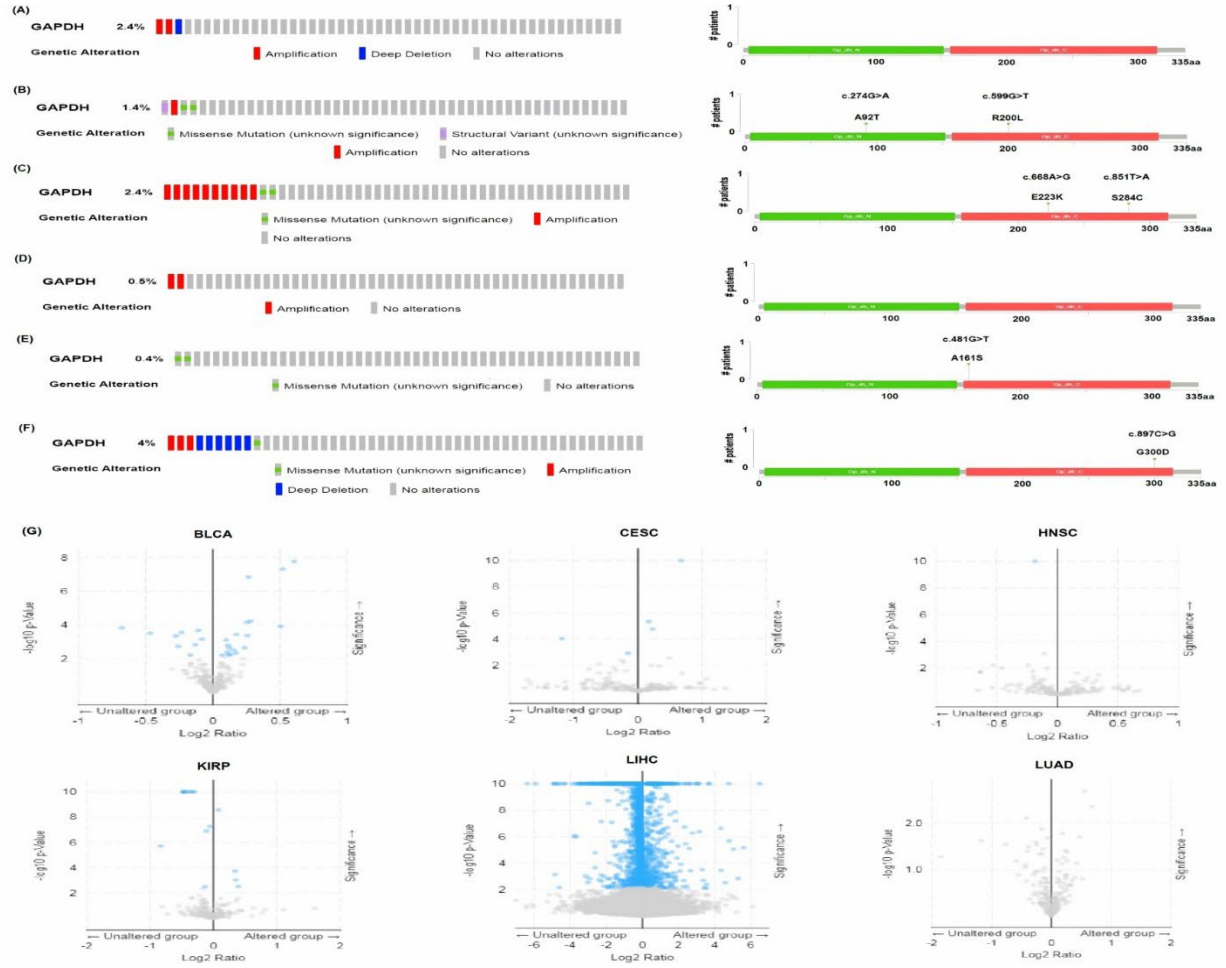

Figure 7. Analysis of genetic alterations in GAPDH across different TCGA datasets. (A) BLCA dataset, (B) CESC dataset, (C) HNSC dataset, (D) KIRP dataset, (E) LIHC dataset, and (F) LUAD dataset.

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

Firstly, GEPIA was used to identify GAPDH co-express genes in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. Analysis results showed that TPI1, GAPDHP1, PHB2, ALDOA, YBX3, ENO1, PKM, MRPL51, USP5, and NCAPD2 are the top nine co-express genes with GAPDH (**Figure 8A**). We further processed these GAPDH-enriched genes for pathway analysis using the DAVID tool. Results have shown that GAPDH-enriched genes were significantly involved in different pathways including "Fructose and mannose metabolism," "Glycolysis/Gluconeogenesis," "Biosynthesis of amino acids," "Carbon metabolism," etc. (**Figure 8B**).

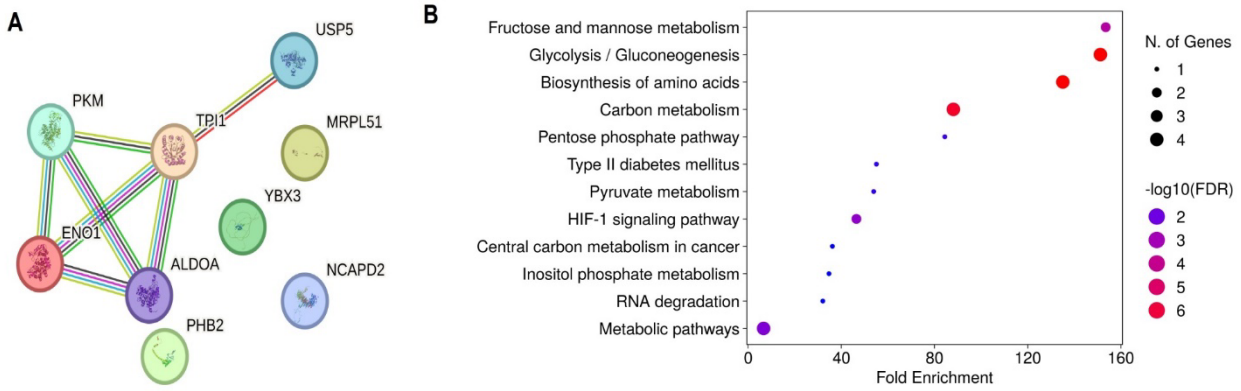

Figure 8. Construction of a protein-protein interaction (PPI) network and pathway analysis of genes enriched with GAPDH. (A) PPI network displaying the interactions among genes enriched with GAPDH, and (B) Pathway analysis illustrating the functional pathways associated with genes enriched with GAPDH.

3.7. GAPDH and infiltrating 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 our study, TIMER was used to calculate the Spearman correlation between the expression of GAPDH and the level of CD8$^+$ T cells. The results demonstrated a significant ($P < 0.05$) positive correlation between the expression of GAPDH and CD8$^+$ T immune cell level in BLCA, while a significant ($P < 0.05$) negative correlation was observed in CESC, HNSC, KIRP, LIHC, and LUAD (**Figure 9**).

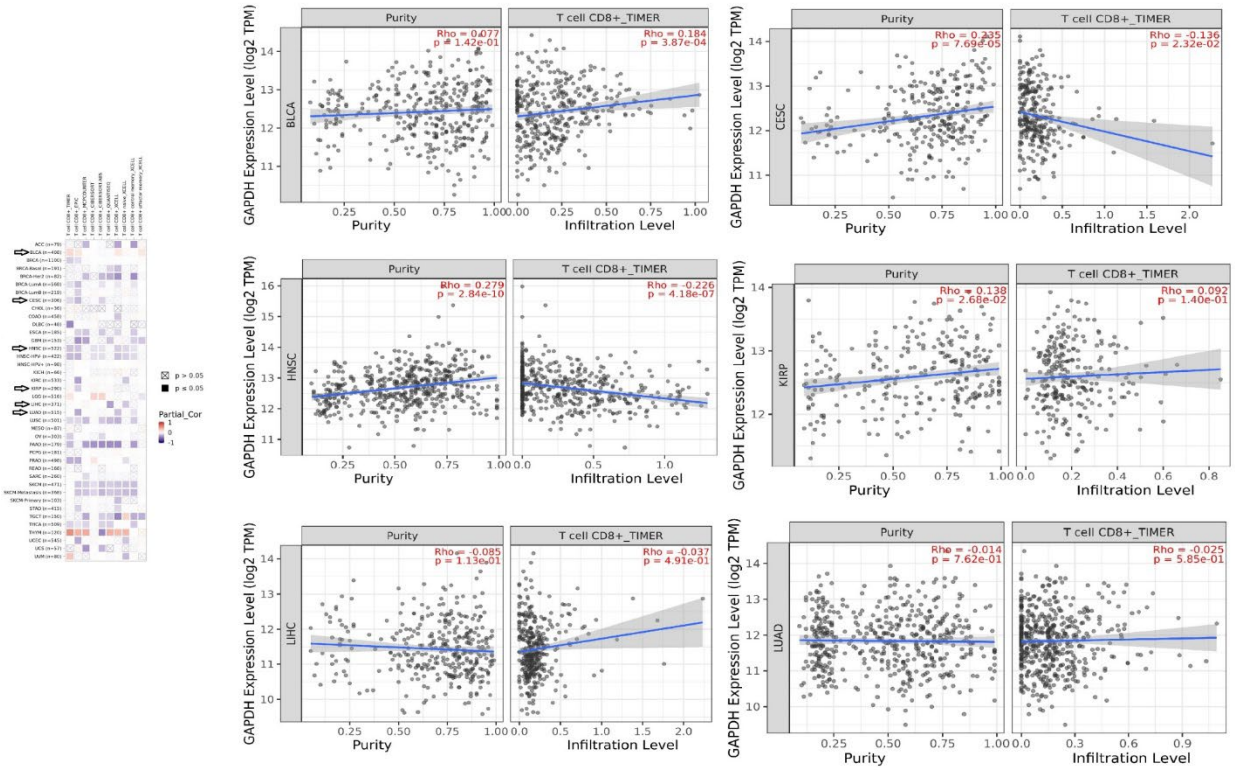

Figure 9. Correlational analysis of GAPDH expression and levels of CD8$^+$ T cells in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD using TIMER. $P < 0.05$ was considered indicative of statistically significant results.

3.8. GAPDH-associated drugs

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

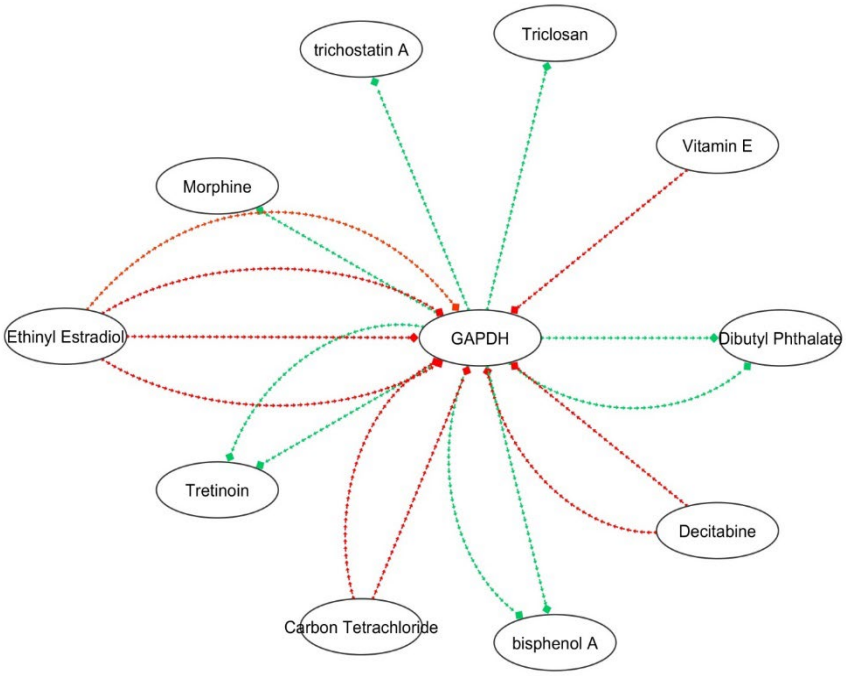

Figure 10. Network of drugs targeting GAPDH. Red color indicates drugs that can increase GAPDH expression, and green arrows represent drugs that can decrease GAPDH expression. The numbers of arrows denote the reference count of selected drugs.

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 explicitly catalyzed the conversion of glyceraldehyde-3-phosphate (G-3-P) to 1,3-diphosphoglycerate [31]. Besides, GAPDH also contributes to numerous other cellular functions, for instance, it participates in the export of nuclear tRNA, DNA repair, DNA replication, exocytosis, endocytosis, cytoskeletal organization carcinogenesis, and cell death [32,33].

Although GAPDH is commonly utilized 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, prostatic cancer, liver cancer, colorectal cancer, pancreatic cancer, gastric cancer, melanoma, and bladder cancer [35]. Increased levels of GAPDH have also been confirmed as a pro-apoptotic agent by Nakajima *et al.* [36]. Such variation in the expression of GAPDH in different cancer subtypes suggested its inconsistent role in the determination of 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 immune cells level. 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 immune cell level. By exploring these relationships, we aimed to enhance our understanding of the potential role of GAPDH in cancer development and its significance as a biomarker in different cancer types.

We observed that GAPDH was up-regulated 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, our study focused primarily on BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. Furthermore, we found that GAPDH was significantly overexpressed ($P < 0.05$) in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients across different clinicopathological features, including cancer stages, patient races, and nodal metastasis statuses, when 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. Moreover, an increased GAPDH expression in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients of different nodal metastasis statuses has implied 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, we performed a correlation analysis using the MEXPRESS database to examine the relationship between GAPDH and its expression levels. Results revealed an expected significantly negative correlation between its expression and promoter methylation level. Collectively, our findings suggest that GAPDH expression regulation in CESC, HNSC, KIRP, LIHC, and LUAD may be significantly influenced by GAPDH hypomethylation. However, further experimental studies on a larger scale are necessary to validate and expand upon these findings.

Several biomarkers have been identified so far for the diagnosis and prognosis of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. For example, Recently, Brisuda *et al.* identified circulating tumor DNA (ctDNA) as a novel biomarker of BLCA [37]. Berger *et al.* [38] identified various mutated genes as novel biomarkers of CESC by analyzing Gene GEO datasets. Song *et al.* [39] developed a long noncoding RNA-microRNA-mRNA network in CESC using GEO datasets and provided novel insights into CESC Biology. Sawyers *et al.* published a review article in which they shed light on 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 so far including VHL [41], VEGF [42], CAIX [43], and HIF1a/2a [44]. Furthermore, the diagnostic and prognostic potential of the different genes including TTF-1, p63, CK5/6, Napsin A, SPATS2, and ST6GALNAC1 have also been well identified in LUAD by a previous study [45]. However, there is a lack of generalization of any biomarkers in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients with diverse clinicopathological features. Herein, we observed a significant up-regulation of GAPDH expression in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients

with various clinicopathological features compared to the control group. Additionally, our analysis of GAPDH promoter methylation level, 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]. Our study revealed intriguing correlations between GAPDH expression and $CD8^+$ T immune cells in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD. These findings suggest that GAPDH might play a role in modulating the immune response and contribute to the development of these cancers.

In our 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," "Carbon metabolism," etc. In addition, we have also identified a few potential drugs that could help to prevent BLCA, CESC, HNSC, KIRP, LIHC, and LUAD by controlling GAPDH expression.

5. Conclusion

In summary, our study has identified the diagnostic significance of GAPDH in BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients with diverse clinicopathological features. We have also assessed the prognostic values of GAPDH and established correlations with its expression, which could potentially aid in predicting the prognosis of BLCA, CESC, HNSC, KIRP, LIHC, and LUAD patients using GAPDH as a prognostic marker. However, further experimental investigations are warranted before translating these findings into clinical implications.

Disclosure statement

The author declares no conflict of interest.

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