**Journal of Information Systems Engineering and Management**
2025, 10(58s)
e-ISSN: 2468-4376
https://www.jisem-journal.com/

**Research Article**

# Significance of Altered Enzyme Expression as Potent Biomarkers for the Early Diagnosis of Gastric: Bioinformatic and Biochemical Studies

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**ARTICLE INFO**

Received: 18 Dec 2024

Revised: 10 Feb 2025

Accepted: 28 Feb 2025

**ABSTRACT**

Enzymes play a crucial role in cellular processes, influencing both function and metabolism. This study aimed to elucidate the impact of altered enzyme expression in gastric cancer on patient survival rates and the underlying pathways involved. Data from The Cancer Genome Atlas (TCGA) and other relevant sources was utilized to analyze changes in the expression of all known enzymes. Cox regression analysis explored the relationship between enzyme expression and disease prognosis, incorporating clinical data to construct a predictive model for patient survival. A co-expression network was used to identify pathways associated with these enzymes. To validate the findings, samples from gastric cancer patients (GCP) and adjacent normal tissues were analyzed using RT-qPCR. The expression of the 1818 enzyme was notably identified in both cancerous and normal TCGA samples. Among these, 56 enzymes exhibited significant overexpression in cancer samples, while 158 genes showed significant decreases in expression. These findings highlight the substantial changes in enzymes associated with gastric cancer, potentially affecting its development and progression. The research also revealed that upregulated genes were mainly associated with cell proliferation, metastasis, and DNA repair, while downregulated genes were mainly involved in metabolic pathways e.g. xenobiotic metabolism, fatty acid, and glucose metabolism. The study revealed a remarkable correlation between the modified expression of heparan sulfate glucosamine 3-O-Sulfotransferase 2 (HS3ST2), dehydrogenase/reductase member 1 (DHRS1), and phosphodiesterase 4C (PDE4C). These results suggest that these enzymes could be considered important diagnostic and prognostic biomarkers for the early detection of gastric cancer.

**Keywords:** Gastric Cancer; Biomarker; Gene Expression; Cancer Prognosis; Enzyme Profile.

## INTRODUCTION

Gastric cancer (GC) poses a significant health threat, ranking as the fifth most prevalent cancer globally and the second leading cause of cancer-related deaths [1]. The incidence of GC varies across regions, with higher rates observed in countries like Japan, China, and Russia [2]. Also, Ardabil province in Iran has experienced a notable rise in stomach cancer cases in recent decades [3]. Despite advancements in diagnosis and treatment, many GC cases are still detected at advanced stages, leading to low 5-year survival rates. Studies have highlighted the role of altered cellular metabolism in cancer development and progression, influencing cellular functions and promoting cell proliferation [4-8].

The tumor microenvironment, characterized by poor vascularity, leads to nutrient and oxygen deprivation, forcing cancer cells to adapt their metabolic pathways and enzyme activity to survive under

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harsh conditions [9-12]. Extensive research has explored the relationship between enzymes and various types of cancer. Many enzymes, including carboxylases, dehydrogenases, and kinases, play crucial roles in maintaining cellular homeostasis and survival [13-20]. Specifically, enzymes such as pyruvate kinase M2 (PKM2) and phosphoglycerate kinase 1 (PGK1) are highly expressed in cancer cells, contributing to their growth [21, 22]. The involvement of 3-Hydroxy-3-Methylglutaryl-CoA Synthase 1 (HMGCS1) in gastric cancer progression has also been confirmed [23].

The main objective of this study is to identify key enzymes that are associated with the development and malignancy of gastric cancer. These enzymes have the potential to serve as therapeutic targets or diagnostic markers. The researchers utilized data from TCGA to analyze changes in enzyme expression between cancerous and normal samples. They also correlated protein expression with patient mortality rates to establish their significance in gastric cancer prognosis. Additionally, a co-expression network was employed to identify pathways related to these enzymes. The findings were further validated using RT-qPCR analysis of gastric cancer and adjacent normal tissues. In summary, this passage emphasizes the global importance of gastric cancer, highlights the role of altered metabolism and enzymes in its progression, and outlines the study's objective to identify key enzymes and pathways associated with gastric cancer for potential therapeutic and diagnostic applications.

## MATERIALS AND METHODS

### Data sources

The study utilized the TCGAbiolinks package to investigate enzymes associated with gastric cancer progression using RNAseq data from the TCGA database. To ensure data quality, genes with minimal or zero expression in 70% of samples were filtered out using the edgeR package [24]. The data were then normalized using the TMM method and logarithmically transformed with a base of 2 [25], facilitated by the limma package. The resulting expression profile was used for the analysis. Clinical data for gastric cancer was downloaded and utilized to categorize samples and correlate gene expression with survival outcomes. The study included 32 normal samples and 375 cancer samples at different stages of the disease according to TCGA data. To identify and extract enzyme information, the HUGO database (https://www.genenames.org/) was utilized. Additionally, for identifying pathways associated with candidate enzymes, the EnrichR database (https://maayanlab.cloud/Enrichr) and the Msigdb repository were employed.

### Survival analysis, co-expression network, and data enrichment

This study aimed to investigate the association between the expression of candidate enzymes and the survival outcomes of patients diagnosed with gastric cancer. To ensure the robustness of our analysis, we carefully filtered the clinical data obtained from the TCGA database. Patients with incomplete or very short survival data, indicated by 'NA', '0', or '1' days to live, were excluded from the study. Furthermore, we specifically focused on samples where tumors were present at the time of death, resulting in a refined dataset comprising 293 samples. Among these samples, 59 patients had unfortunately passed away, while 234 were still alive, serving as the basis for our subsequent analysis.

The normalized matrix was used to evaluate the impact of candidate gene expression on patient survival. By standardizing the expression levels of each gene across all samples using Z-scores, we ensured comparability. Subsequently, Cox regression analysis was performed using the Z-scores within the same sample [26], to assess the relationship between gene expression and survival outcomes. To validate the findings, Kaplan-Meier survival curves were generated [27]. For further analysis, the cancer samples were categorized into two groups, 'high' and 'low,' based on the median expression of candidate enzymes. This categorization provided insights into the differential expression patterns of these genes.

In addition, the pathways associated with these candidate enzymes were studied. To construct a co-expression network, the normalized expression matrix was utilized from the previous steps [28]. By applying expression correlation tests between the candidate enzyme's expression level and all other

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e-ISSN: 2468-4376
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genes, we identified genes with expression levels exceeding 0.5 and a significance level of P<0.01 for inclusion in the co-expression network. This network enables the investigation of potential relationships and interactions among genes.

### Drug resistance and sensitivities

To explore the association between candidate gene expression and drug resistance and sensitivity in cancer, the data from the GDSC database were utilized. This database provided valuable information, including expression data for 1106 cancer cell lines and $IC_{50}$ values for 190 drugs. Data were downloaded using the PharmacoGX package. To assess the relationship between the expression levels of candidate genes and the $IC_{50}$ values of drugs, a correlation test was conducted using the tools available in the PharmacoGX package. The analysis ensured that each lncRNA had a sufficient degree of freedom, with more than 30 observations for each lncRNA, enabling reliable conclusions regarding drug resistance and sensitivity.

### Sample collection

We collected a total of 25 gastric cancer samples and 25 adjacent normal tissue samples from the Iran Tumor Bank. In compliance with the regulations of the Ministry of Health, Treatment, and Medical Education of Iran, all bioethical considerations were thoroughly reviewed and approved by the Ethics Committee of the University of Tabriz (IR.TABRIZU.REC.1403.019). To provide comprehensive clinical information, a pathologist meticulously summarized the disease status of each cancer sample, which can be found in Table 1. Additionally, to maintain sample integrity, all collected samples were immediately stored in liquid nitrogen until further use.

**Table 1**. Characteristics of studied samples.

| Label | Age | Gender | stage | TNM.T | TNM.N |
|---|---|---|---|---|---|
| T1 | 68 | Female | II | T1 | N+ |
| T2 | 52 | Male | I | T1 | N+ |
| T3 | 49 | Female | III | T2 | N+ |
| T4 | 60 | Female | II | T3 | No |
| T5 | 76 | Male | IV | T3 | N+ |
| T6 | 59 | Female | II | T2 | No |
| T7 | 61 | Female | IV | T1 | N+ |
| T8 | 58 | Male | IV | T3 | N+ |
| T9 | 47 | Male | I | T1 | No |
| T10 | 69 | Male | III | T3 | N+ |
| T11 | 48 | Female | II | T2 | No |
| T12 | 64 | Male | II | T1 | No |
| T13 | 53 | Male | II | T2 | N+ |
| T14 | 64 | Male | IV | T3 | N+ |
| T15 | 51 | Male | III | T3 | N+ |
| T16 | 49 | Female | I | T1 | No |
| T17 | 63 | Female | IV | T3 | N+ |

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| | | | | | |
|---|---|---|---|---|---|
| T18 | 61 | Male | II | T2 | No |
| T19 | 61 | Male | II | T2 | No |
| T20 | 39 | Female | III | T2 | N+ |
| T21 | 47 | Female | I | T1 | No |
| T22 | 56 | Male | IV | T4 | N+ |
| T23 | 61 | Male | II | T3 | No |
| T24 | 72 | Female | II | T2 | N+ |
| T25 | 81 | Male | III | T2 | N+ |

TNM.T: *Tumor, Nodes, and Metastasis/tumor status*; TNM.N: *Tumor, Nodes, and Metastasis/nodes status*; N+: *refers to nodes involved*; No: *refers to nodes not involved*.

### RNA extraction, cDNA synthesis, and RT-qPCR

RNA extraction from the samples was performed using TRIzol (Genius Gene) after three washes with PBS to eliminate contamination and necrotic cells [29]. Subsequently, DNase I (Sina Colon) was utilized to remove any DNA contamination. The cDNA synthesis was carried out using Amplicence's kit following the manufacturer's instructions. Specific primers (Table 2) for HS3ST2, DHRS1, and PDE4C were designed using the Primer-BLAST tool [30]. To quantify the expression of the mentioned genes in both cancer and normal samples, RT-qPCR was performed with specific primers and SYBR GREEN (Amplicon). The gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [31], and GAPDH served as the internal control for normalization [32, 33].

**Table 2**. The primers used in this study were as follows.

| Gene name | Forward primer | Reverse primer |
|---|---|---|
| HS3ST2 | GGGCAAATCAAAAGGGAGAAC | ACGGAAGAGATGAGCAGAAGA |
| DHRS1 | CATTTGGACTTGGTGGTTCGT | GACACACAGCAGAGGGCTT |
| PDE4C | AGTTTCCCCTCTGTAACCTG | AGATAAATGCTCAGTACCCCAA |
| GAPDH | TGCCGCCTGGAGAAACC | TGAAGTCGCAGGAGACAACC |

### Statistics and software

All preprocessing and data analysis were conducted using R language (version 4.0.2). GraphPad software (v8) was utilized for generating and displaying graphs. Differential expression analysis was performed using the linear model method, and the significance levels were determined using multiple hypothesis tests [34]. A significance level of FDR<0.05 was considered for all analyses. To visualize the co-expression network and the relationship between genes and identified enzymes, Cytoscape (v4) was employed. The logRank method was utilized to assess the significance of survival tests. Additionally, receiver operating characteristic curve (ROC) and Kaplan-Meier (K-M) curves were utilized to investigate the association between the expression of candidate enzymes and the death rate of patients, as well as to evaluate the potential of candidate enzymes as diagnostic biomarkers [35].

## RESULTS

### Enzyme expression changes

A comprehensive analysis of TCGA was conducted to identify key enzymes involved in the development of gastric cancer. The study compared the expression levels of all enzymes in cancer samples with their

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corresponding normal samples. Out of the 1854 enzymes examined, 1818 enzymes showed expression in both cancerous and normal TCGA samples. Upon initial evaluation, 56 enzymes were found to be significantly upregulated in cancer samples, meeting the criteria of logFC>1 and FDR<0.01 (Figure 1; A). Conversely, 158 genes exhibited significantly reduced expression, characterized by logFC<1 and FDR<0.01 (Figure 1; B). These alterations in enzyme expression could potentially play a role in the development and progression of gastric cancer.

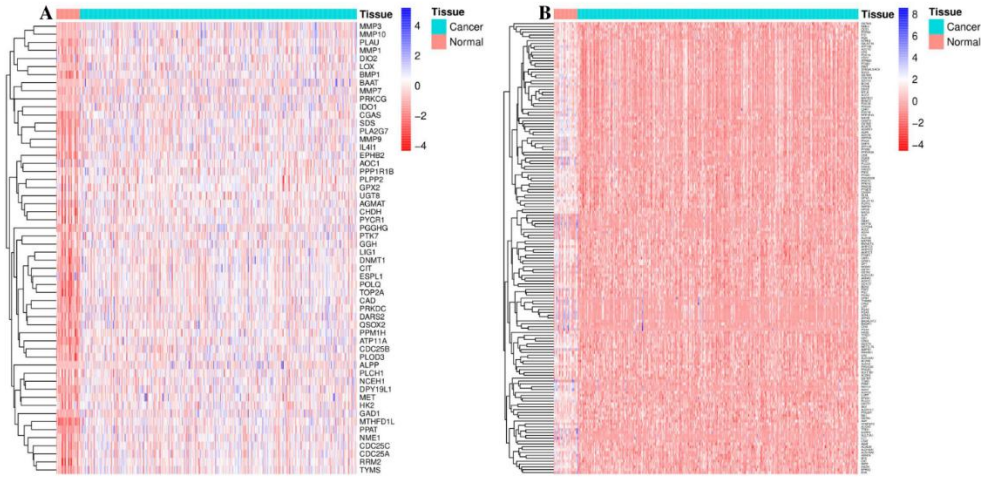


**Figure 1**. Heatmaps of studied genes and enzymes.

In order to gain a deeper understanding of the identified enzymes, their associated pathways were thoroughly assessed. It was observed that the majority of upregulated genes were involved in pathways linked to cell proliferation (E2F targets and G2-M checkpoint), metastasis (Epithelial Mesenchymal Transition (EMT)), and DNA repair (Figure 2; A). On the other hand, downregulated genes were primarily associated with metabolic pathways such as xenobiotic metabolism, fatty acid metabolism, glycolysis, and bile acid metabolism (Figure 2; B). These findings indicate that the identified enzymes play crucial roles in key pathways within cancer cells, potentially contributing to the malignancy of gastric cancer through these specific mechanisms.

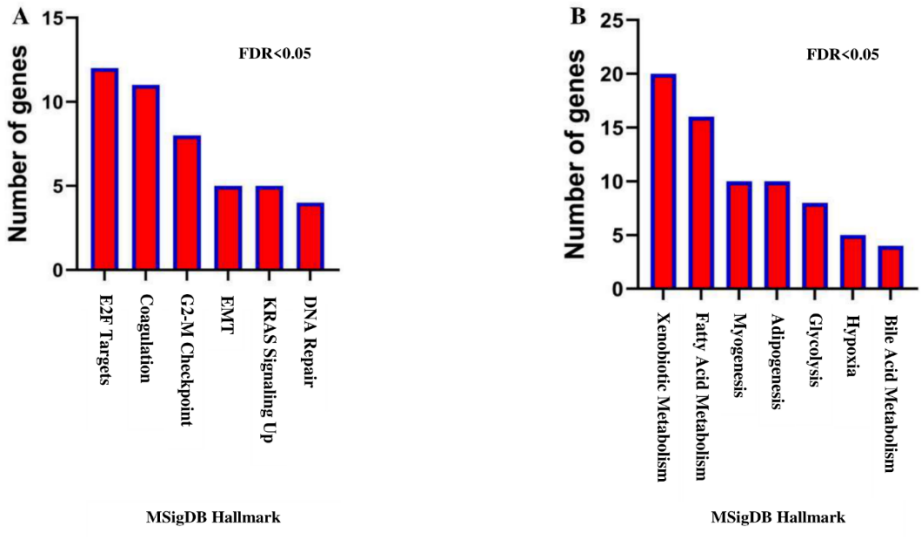


**Figure 2.** The studied enzymes with their related pathways.

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### Prognostic value of enzyme expression in GC

Univariate Cox regression analysis revealed that among the 56 upregulated enzymes (Table 3), elevated expression of Carbohydrate Sulfotransferase 1 (CHST10), HS3ST2, and Protein C (PROC) was linked to poor prognosis.

**Table 3**. Cox regression analysis outputs for desired genes.

| Gene | Beta | LogRank | HR | HRlower | HRupper |
|---|---|---|---|---|---|
| CHST10 | 0.351944706 | 0.011569924 | 1.421829903 | 1.080413519 | 1.87113567 |
| HS3ST2 | 0.25204829 | 0.038658651 | 1.286658169 | 1.011704022 | 1.636337513 |
| PROC | 0.303116909 | 0.029456173 | 1.354072758 | 1.030214116 | 1.77973977 |
| ALDH3A2 | -0.322611252 | 0.012147884 | 0.724255352 | 0.566021932 | 0.926723481 |
| CHAC1 | -0.365174658 | 0.013380511 | 0.694075415 | 0.51875893 | 0.928640748 |
| DHRS1 | -0.359165309 | 0.01611014 | 0.698258914 | 0.521618066 | 0.934717454 |
| NMRK1 | -0.288615693 | 0.031487662 | 0.749300111 | 0.575385637 | 0.975781495 |
| PDE4C | -0.428292215 | 0.003439958 | 0.651620974 | 0.487415892 | 0.87114495 |

On the other hand, the study revealed that increased expression of six enzymes, namely Aldehyde dehydrogenase 3 family member A2 (ALDH3A2), ChaC glutathione-specific gamma-glutamyl cyclo transferase 1 (CHAC1), DHRS1, Nicotinamide riboside kinase 1 (NMRK1), PDE4C, and Phospholipase A2 group IVF (PLA2G4F), was associated with a positive prognosis for patients. To validate these findings, Kaplan-Meier survival curves were utilized. Notably, gastric cancer samples with elevated CHST10, HS3ST2, and PROC expression displayed lower survival rates (Figure 3). Conversely, higher expression of ALDH3A2, CHAC1, DHRS1, NMRK1, PDE4C, and PLA2G4F was linked to reduced patient mortality (Figure 3). These results emphasize the potential of CHST10, HS3ST2, PROC, ALDH3A2, CHAC1, DHRS1, NMRK1, PDE4C, and PLA2G4F as prognostic biomarkers among the identified enzymes. Additionally, these enzymes may play a vital role in the development of gastric cancer. Further analysis focused on three enzymes - HS3ST2, DHRS1, and PDE4C - which have received less attention in the context of gastric cancer.

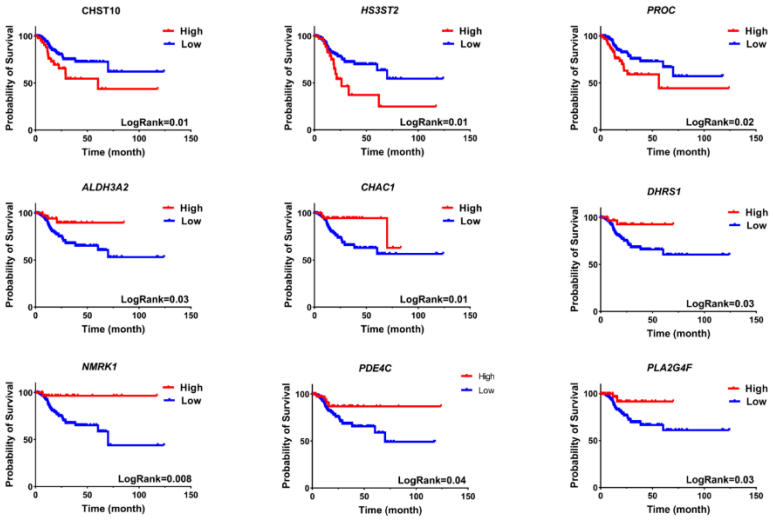


**Figure 3**. Kaplan-Meier survival curves for target genes.

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https://www.jisem-journal.com/

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## DISCUSSION

Cell metabolism changes have garnered increasing interest due to their association with cancer pathology mechanisms and their role in tumor occurrence and development [36, 37]. These changes disrupt anabolic and catabolic processes, jeopardizing the homeostasis of cell rest and proliferation. Reprogrammed metabolic activities contribute to cancer initiation and progression. Cancer cells require ample energy and biosynthesis for division, making the identification of relevant genes crucial for developing strategies to prevent cancer, inhibit cell growth, and mitigate metastasis [38, 39].

Studies have shown that the overexpression of HS3ST2, 3B, and 4 has a positive impact on breast cancer survival and proliferation. This overexpression is accompanied by increased activation of key signaling molecules such as c-Src, Akt, and NF-B. Furthermore, it leads to enhanced regulation of protective proteins like Survivin and X-linked inhibitor of apoptosis protein (XIAP) [40]. Conversely, lower expression levels of PDE4C and DHRS1 genes have been associated with poor cognition and the progression of cancer. A study revealed that DHRS1 expression levels were significantly reduced in hepatocellular carcinoma (HCC) compared to normal tissues, and this decrease was associated with a poor prognosis [41]. Another study has shown that PDE4C promoter hypermethylation is linked to low PDE4C expression. High-grade glioma patients with hypermethylation and low PDE4C expression tend to have an unfavorable prognosis. These findings suggest that both PDE4C promoter methylation and PDE4C expression can serve as prognostic biomarkers for glioma patients. Furthermore, PDE4C-induced apoptosis in glioma is believed to involve the cAMP-P53 pathway [42].

Our study revealed the dysregulation of multiple enzymes in gastric cancer. Specifically, the expression of ALDH3A2, CHAC1, DHRS1, NMRK1, PDE4C, and PLA2G4F significantly increased in GC patients, whereas higher expression levels were observed for CHST1, HS3ST2, and PROC. These findings suggest that the expression levels of CHST1, HS3ST2, PROC, ALDH3A2, CHAC1, DHRS1, NMRK1, PDE4C, and PLA2G4F can serve as prognostic biomarkers for gastric cancer. Furthermore, these enzymes may contribute to the malignancy of stomach cancer. To further our analysis, we focused on three enzymes, HS3ST2, DHRS1, and PDE4C, which were less prominent in GC (Figure 4).

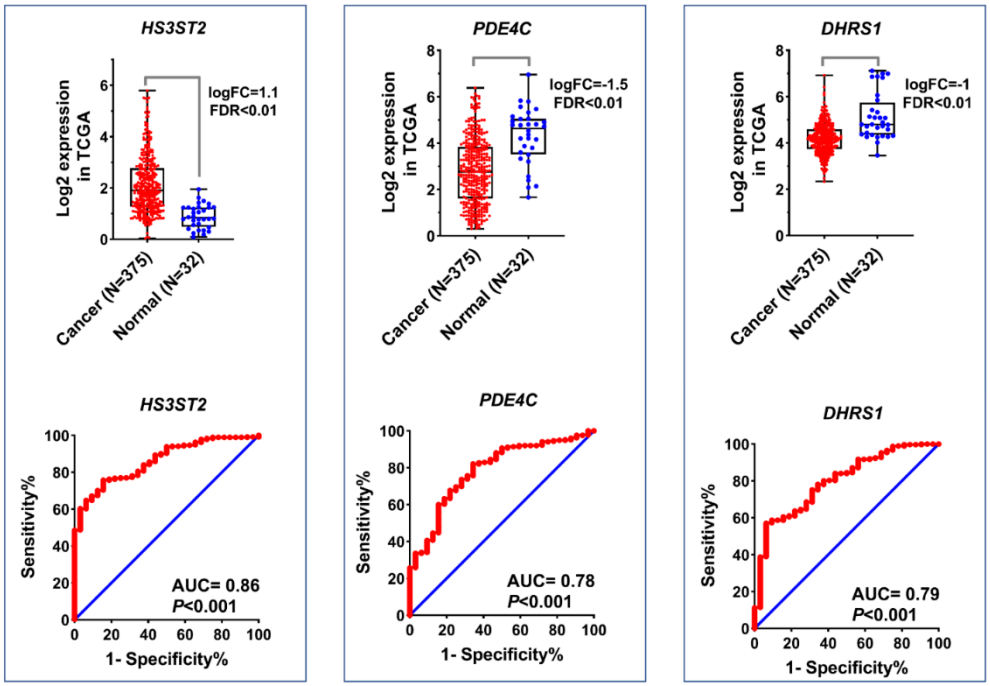


**Figure 4**. Genes with positive prognostic value were identified in this study.

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e-ISSN: 2468-4376
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The co-expression network analysis was utilized to explore pathways associated with candidate enzymes. HS3ST2 expression levels exhibited significant correlations with 50 genes, most of which are involved in malignancy-related pathways like KRAS, inflammation, and mTORC1. Additionally, upregulation of HS3ST2 is associated with lymph node metastatic colorectal cancer [43]. DHRS1 expression levels showed significant correlations with 39 genes related to fatty acid metabolism, bile acid metabolism, oxidative phosphorylation, xenobiotic metabolism, and the p53 pathway (Figure 5). PDE4C expression also displayed significant correlations with 38 genes. These findings suggest that the candidate enzymes may contribute to the pathogenesis of gastric cancer through the mentioned pathways.

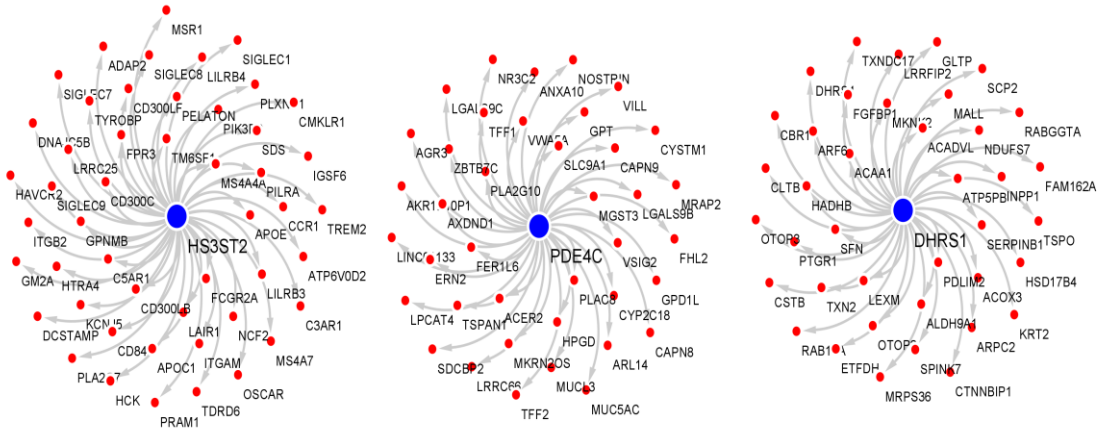


**Figure 5**. The co-expression network analysis for candidate enzymes.

## CONCLUSION

GC remains a significant global health challenge. Despite advancements in diagnostic and therapeutic approaches, many GC cases are detected at advanced stages. Altered cellular metabolism and patterns of enzyme expression are recognized as critical factors in cancer development and progression, influencing various cellular functions and promoting proliferation. This study aimed to identify key enzymes involved in the progression of gastric cancer and evaluate their potential as therapeutic targets or diagnostic markers. Utilizing data from TCGA, we analyzed enzyme expression changes between cancerous and normal tissues.

Our findings revealed significant alterations in the expression of enzymes in gastric cancer. Enzymes such as HS3ST2, DHRS1, and PDE4C emerged as significant prognostic biomarkers. Overexpression of HS3ST2 was associated with poor prognosis and linked to pathways involving KRAS, inflammation, and mTORC1. In contrast, DHRS1 and PDE4C were connected to pathways related to metabolism and oxidative phosphorylation. These findings underscore the importance of these enzymes as potential biomarkers for gastric cancer prognosis. Their involvement in crucial metabolic and signaling pathways highlights their roles in the malignancy of gastric cancer. This study provides a foundation for further research into the therapeutic targeting of these enzymes and the development of diagnostic tools to improve gastric cancer outcomes.

### Abbreviations

| | |
|---|---|
| ALDH3A2 | Aldehyde dehydrogenase 3 family member A2 |
| CHAC1 | ChaC glutathione-specific gamma-glutamyl cyclo transferase 1 |
| CHST10 | Carbohydrate Sulfotransferase 1 |

| | |
|---|---|
| DHRS1 | Dehydrogenase/reductase member 1 |
| DNA | Deoxyribonucleic Acid |
| EMT | Epithelial Mesenchymal Transition |
| GC | Gastric cancer |
| GCP | Gastric cancer patients |
| HCC | hepatocellular carcinoma |
| HMGCS1 | 3-Hydroxy-3-Methylglutaryl-CoA Synthase 1 |
| HS3ST2 | Heparan sulfate glucosamine 3-O-Sulfotransferase 2 |
| NMRK1 | Nicotinamide riboside kinase 1 |
| PDE4C | Phosphodiesterase 4C |
| PGK1 | Phosphoglycerate kinase 1 |
| PKM2 | Pyruvate kinase M2 |
| PLA2G4F | Phospholipase A2 group IVF |
| PROC | Protein C |
| ROC | Receiver operating characteristic curve |
| TCGA | The Cancer Genome Atlas |
| XIAP | X-linked inhibitor of apoptosis protein |
| | |

**Acknowledgments**

Thanks to Genius Gene Center for assisting in laboratory work and data analysis.

**Author contributions**

**Babak Ghobari:** Writing original draft; Investigation; Methodology; Data curation; Formal analysis; **Gholamreza Dehghan:** Conceptualization, Project administration; Supervision; Writing - review & editing; **Maryam Peymani:** Supervision, validation, and visualization Reviewing, editing, and proofing, **Amirnader Emami Razavi:** Resources, Visualization; Methodology.

All the authors have read and approved the final manuscript.

**Funding**

Not available.

**Data availability**

The corresponding author can provide supporting and raw data upon a reasonable request.

**Declarations**

**Ethics approval and consent to participate**

The bioethical issues related to this study underwent a thorough review and were approved by the Ethics Committee of the University of Tabriz (IR.TABRIZU.REC.1403.019), following the criteria set by the Ministry of Health and Medical Education of Iran. Written consent forms were obtained from all individuals who participated in the study. It is worth mentioning that the samples used in this study were previously utilized in another research project. All techniques adhered to the pertinent standards

and regulations, and written informed permission was secured from all participants or their representatives.

### Consent for publication

Not applicable.

### Competing interests

The authors state that they have no known financial interests or personal relationships that could have influenced the work reported in this paper.

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