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Identification of gastric cancer biomarkers through *in-silico* analysis of microarray based datasets

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ABSTRACT

Gastric cancer is among the most prevalent cancers worldwide including in Pakistan. Late diagnosis of gastric cancer leads to reduced survival. The present study aimed to investigate biomarkers for early diagnosis and prognosis of gastric cancer. For this purpose, the ten microarray-based gene expression datasets (GSE54129, GSE79973, GSE161533, GSE103236, GSE33651, GSE19826, GSE118916, GSE112369, GSE13911, and GSE81948) were retrieved from GEO database and analyzed by GEO2R to identify differentially expressed genes. Datasets were arranged in subsets of different dataset combinations to identify common DEGs. The gene ontology and functional pathway enrichment analysis of common DEGs was performed by DAVID tool. Pan-cancer analysis was conducted by UALCAN database. Survival analysis of common DEGs was done by Kaplan-Meier plotter. A total of 71 common DEGs were identified in different combinations of datasets. Among them, only 5 DEGs namely ATP4B, ATP4A, CCKBR, KCNJ15, and KCNJ16 were detected to be common in all the datasets. The GO and pathway analysis represented that the identified DEGs are involved in gastric acid secretion and collecting duct acid secretion pathways. Further expression validation of these five genes using three additional datasets (GSE31811, GSE26899, and GSE26272) confirmed their differential expression in gastric cancer samples. The pan-cancer analysis also revealed aberrant expression of DEGs in various cancers. The survival analysis showed the association of these 5 DEGs with poor survival of gastric cancer patients. To conclude, this study revealed a panel of 5 genes, which can be employed as diagnostic and prognostic biomarkers of gastric cancer patients.

1. Introduction

Gastric cancer (GC) ranks as the fifth most common cancer and the third leading cause of cancer-related mortality worldwide [1]. It is a malignant tumor that initiates in the gastric mucosal epithelium and is one of the most common cancers [2]. In developed countries, gastric cancer is more frequent and more likely to be diagnosed in males than females [3]. According to the World Health Organization (WHO), the gastric cancer rate increases annually by up to 1.8 million cases and is estimated to enhance the death rate by up to 1.3 million by 2040 [4] It is highly prevalent in Asian countries with twice the age-adjusted incidence rate per 100,000 relative to other regions like Europe [5]. Whereas, in South Asia 99,399 new gastric cancer cases were reported in 2019 [6]. In Pakistan, according to the WHO fact sheet, the mortality rate has reached 4044 deaths and 2.97 per 10,000 age-adjusted death rate [7]. Gastric cancer pathogenesis is multifactorial and involves the complex interplay of various risk factors such as genetic predisposition, dietary factors, environmental, and infectious agents, majorly *Helicobacter pylori* infection. These factors affect the stomach lining over a prolonged period, leading to gradual changes that eventually result in cancer development [8].

For early diagnosis of gastric cancer, endoscopy, medical imaging techniques, and biopsy are applied as the predominant methods of cancer screening [9]. Endoscopic screening techniques encompass chromoendoscopy, white light endoscopy, and computed virtual chromoendoscopy, along with other endoscopic methods such as optical coherence tomography, confocal laser endomicroscopy, and endocytoscopy [10]. Imaging techniques include magnetic resonance imaging

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(MRI), upper gastrointestinal barium meal, ultrasonography, and multidimensional spiral computed tomography (MDCT) [10]. For differentiating stomach gastrointestinal cancers (GISTs) from gastric schwannomas (GSs), contrast-enhanced computed tomography (CE-CT) imaging is used [11].

For the treatment of early-stage gastric cancer, endoscopic resection techniques are proven to be fruitful. Whereas, for advanced-stage gastric cancer, chemotherapy and surgery are employed [9]. Diffusion-weighted magnetic resonance imaging (DWI) and spectral computed tomography (CT) have been used in the case of advanced gastric cancer stage for predicting chemotherapy response [11]. Furthermore, metallic nanoparticles emerged as promising drug delivery and clinical imaging agents. Among them, iron oxide nanoparticles (IONps) are widely employed in chemotherapeutic drug delivery. While PEGlated IONps can serve as an anticancer agent [12].

Various biomarkers are in clinical practice for diagnosing gastric cancer including alpha-fetoprotein, carbohydrate antigen (CA) 72-4, 12-5, BCA-225, SLE, hCG, and pepsinogen I/II and among them, carcinoembryonic antigen (CEA) and CA19-9 are the most frequently applied biomarkers of gastric cancer [13]. Besides these, several other molecular and predictive biomarkers including HER2/neu amplification, HER2 overexpression, PD-L1+, and MSI-H of gastric cancer are in clinical trials to ensure their efficacy in targeted therapy [14]. Despite the advancement in diagnostic and therapy strategies like chemotherapy and immunotherapy, and biomarkers detection gastric cancer diagnosis at an early stage poses a challenge due to the late emergence of clinical symptoms and treatment complications [15]. Therefore, there is an urgent need to investigate earlier diagnostic and prognostic biomarkers of gastric cancer.

The high-throughput sequencing technologies and bioinformatic tools aid in the investigation of novel biomarkers and therapeutic targets [16]. Systematic computational approaches like docking, ADMET, and dynamic simulations provide insight into the therapeutic potential of novel biomarkers and guide for developing novel therapeutics. Through the ligand-based approach, the most effective inhibitor model (compound Z1) of CDK-2 was detected [17]. Similarly, the most efficient pharmacophore inhibitor model (Compound S35) of carbonic anhydrase (CA IX) was developed by modeling and MD simulation method [18].

Nowadays, the Gene Expression Omnibus (GEO) database has been extensively used to discover new potential diagnostic and prognostic biomarkers of various cancers. It provides insights into the genetic alterations involved in cancer progression and development [19]. As a previous study reported diagnostic (CXCL12, FOS, DCN, SOCS3, FOSB and PCK1) and prognostic (FOBS and SPP1) biomarkers of liver cancer by analyzing GEO datasets [20].

The presented study aimed to investigate diagnostic gastric cancer biomarkers via *In-silico* analysis of microarray-based datasets that can be globally applicable for screening GC at an early stage to prevent metastasis. This study involves the identification of common differentially expressed genes (DEGs) among various microarray-based datasets of gastric cancer patients. Analyzing KEGG and GO pathways of DEGs helps to understand the disrupted biological processes in gastric cancer. Further, Kaplan Meir's survival curve analysis then detects the effect of gene expression on patient survival. Together these analyses provide insight into the molecular mechanisms and will be fruitful for detecting potential gastric cancer biomarkers.

2. Methodology

2.1. Microarray datasets mining

The microarray datasets of gastric cancer in humans were retrieved from a freely accessible database named Gene Omnibus (GEO) (available at https://www.ncbi.nlm.nih.gov/geo/) which contains microarray and next-generation sequencing (NGS) genomic datasets [21]. In the present study, ten microarray expression datasets i.e., GSE54129, GSE79973, GSE161533, GSE103236, GSE33651, GSE19826, GSE118916, GSE112369, GSE13911, and GSE81948 related to gastric cancer were retrieved from GEO.

Characteristics (cancerous and normal sample size and populations) of studied datasets are provided in Supplementary Table 1.

2.2. Screening for differentially expressed genes (DEGs)

Identification of DEGs was performed by GEO2R available at (http://www.ncbi.nlm.nih.gov/geo/geo2r). Top genes with logFC-1.0 and p-value<0.05 were considered as DEGs. The datasets were arranged in groups of five, four, and three datasets combination. The Funrich software (http://funrich.org) was used to draw the Venn diagram representing common genes.

2.3. Gene ontology and KEGG pathway enrichment analysis of DEGs

Kyoto Encyclopedia of Genes and Genome (KEGG) pathways enrichment analysis and Gene ontology (GO) of DEGs was performed by DAVID (Database for Annotation, Visualization and Integration Discovery) tool (https://davidbioinformatics.nih.gov/). It gives a wide range of tools for functional annotations and enrichment analysis of genes [22]. A p-value < 0.05 was considered as a cut-off value.

2.4. Pan-cancer expression analysis of the DEGs

UALCAN (https://ualcan.path.uab.edu/) is an interactive web resource for analyzing cancer omics data, providing access to comprehensive TCGA datasets [23]. It allows researchers to explore gene expression, promoter methylation, and survival analyses across various cancers. UALCAN's pan-cancer analysis feature enables users to compare gene expression and methylation profiles across multiple cancer types, offering insights into common and unique molecular mechanisms, and thereby facilitating the identification of potential biomarkers and therapeutic targets. In the present study, this database was utilized to perform expression analysis of DEGs in a pan-cancer view. A p-value cutoff of 0.05 was used to show significant differences.

2.5. Survival analysis

For the determination of DEGs as prognostic biomarker, the impact of gene expression on survival was studied by Kaplan-Meier curve analysis. In the present study, survival analysis was conducted using Kaplan-Meier plotter (https://kmplot.com/analysis/) which is a publicly available database having the capability for performing correlation analysis between gene expression and survival in more than 31 thousand samples of 21 cancer types [24]. Statistical analysis was performed for False Discovery Rate computation and hazard ratio was calculated by Cox regression.

3. Results

3.1. Identification of DEGs

For the investigation of DEGs among the ten selected datasets, the following combination scheme of datasets was applied. In the first combination, five datasets i.e., GSE161533, GSE54129, GSE33651, GSE19826, and GSE79973 were grouped. In this combination, only one common DEG named ESSRG was identified (Fig. 1).

In the second combination, four-four datasets were combined. Among them, the combination of GSE118916, GSE79973, GSE103236, and GSE13911 datasets have only one common DEG namely ESSRG, and in the second group of four datasets i.e., GSE33651, GSE79973, GSE118916, GSE161533 combination one common DEG named RAB31was identified (Fig. 2).

In the third combination, three datasets were grouped making seven

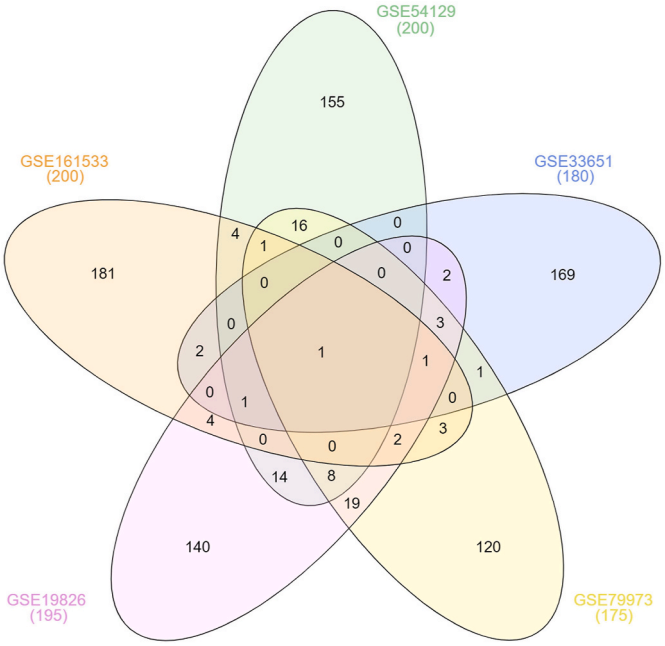

Fig. 1. Identification of DEG among GSE161533, GSE54129, GSE33651, GSE19826, GSE79973 dataset combination. Only 1 gene was noted to be common among 5 datasets.

combinations. In the first combination of GSE13911, GSE581948, GSE79973 datasets, 18 shared DEGs i.e., CWH43, ATP4B, ATP4A, TRIM50, ESRRG, KCNJ16, CCKBR, GHRL, ADH7, AQP4, LINC00982, COL6A3, COL12A1, FGD4, DGKD, SPARC, CKMT2, KCNJ15 were identified. In GSE19826, GSE79973, GSE103236 datasets combination, 08 common DEGs named ESRRG, CCKBR, CKMT2, APOBEC2, THY1, BGN, TIMP1, SPARC were noted. In GSE19826, GSE13911, and GSE103236 datasets, 05 common DEGs including ESRRG, CCKBR, CKMT2, ATP11A, and SPARC were detected. Combination of GSE19826, GSE79973, and GSE118916 datasets reveals 03 shared DEGs ESRRG, TMEM161B, and ADH7. The combination of GSE19826, GSE581948 and GSE103236 showed 23 shared DEGs namely ESRRG, CCKBR, SMIM11A, DUSP19, CKMT2, GPER1, APOBEC2, CNTN3, THY1, ADHFE1, S100A10, CLDN7, ATP11A, BGN, TIMP1, PEBP4, CKB, SCUBE2, SPARC, TTYH3, ITIH5, WNT5A and PNPLA7. In GSE13911, GSE118916, and GSE79973 datasets group, 05 common DEGs (ESRRG, ADH7, CAPN13, SMIM6, and PBLD) were noted. In combined datasets GSE161533, GSE103236, and GSE13911, 07 DEGs including MYOC, SCARA5, PLCXD3, CKS1B, ATAD2, CKMT2, and TNFRSF10B were identified to be common (Fig. 3).

3.2. Functional and pathway enrichment analysis of DEGs

Functional and pathway analysis of DEGs of five datasets combination groups reveals no pathway for one common gene ESSRG (Table 1). The four datasets combination group genes were not detected to be involved in common pathways i.e., no pathway was predicted (Table 2). The pathways analysis of 69 genes from seven three dataset combinations depicted only 07 genes to be involved in two different pathways. Among them, 05 DEGs (ATP4B, ATP4A, CCKBR, KCNJ15, KCNJ16) were involved in the gastric acid secretion pathway and two DEGs (ATP4B, ATP4A) were part of collecting duct acid secretion pathway (Table 3).

3.3. Expression validation of DEGs

For validation analysis of five DEGs, three more datasets i.e., GSE31811, GSE26899, and GSE26272 were retrieved from the GEO database, and all the genes were found to be differentially expressed in three datasets (Table 4).

3.4. Survival analysis of DEGs

Overall survival (OS) analysis of DEGs represents that except KCNJ15 the high expression of ATP4A, ATP4B, CCKBR, and KCNJ16 were significantly (HR $>$ 1.0, logrank P $<$ 0.05) correlated with decreased OS rate in GC patients and thus these genes can be predicted as a good prognostic biomarker of GC (Fig. 4). Whereas Disease-free survival (DFS) analysis depicted that high expression of ATP4A, ATP4B, CCKBR, and KCNJ16 to be significantly (HR $>$ 1.0, logrank P $<$ 0.05) associated with DFS and can be proposed as a good prognostic biomarker (Fig. 5).

3.4.1. Pan-cancer expression analysis of the DEGs

Pan-cancer expression analysis of ATP4B, ATP4A, CCKBR, KCNJ15,

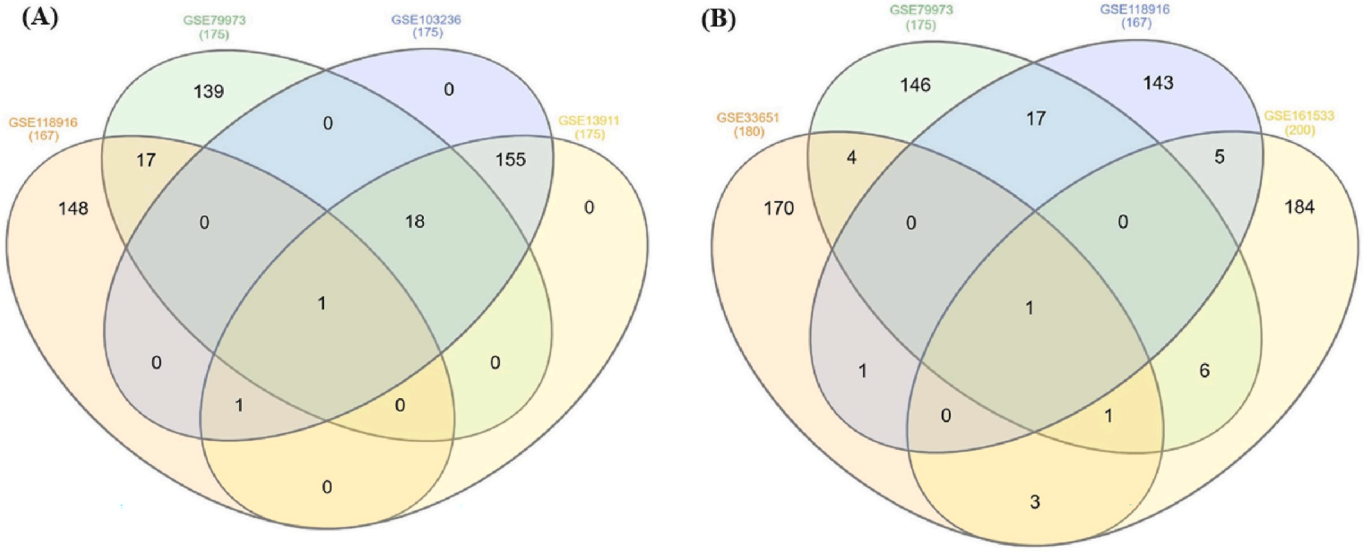

Fig. 2. Identification of DEG among groups of four-four dataset combinations. (A) DEGs analysis among GSE118916, GSE79973, GSE103236, GSE13911 datasets combination. Only 1 gene was noted to be common among these 4 datasets (B) DEGs analysis among GSE33651, GSE79973, GSE118916, and GSE161533 datasets combination. Only 1 gene was noted to be common among these 4 datasets.

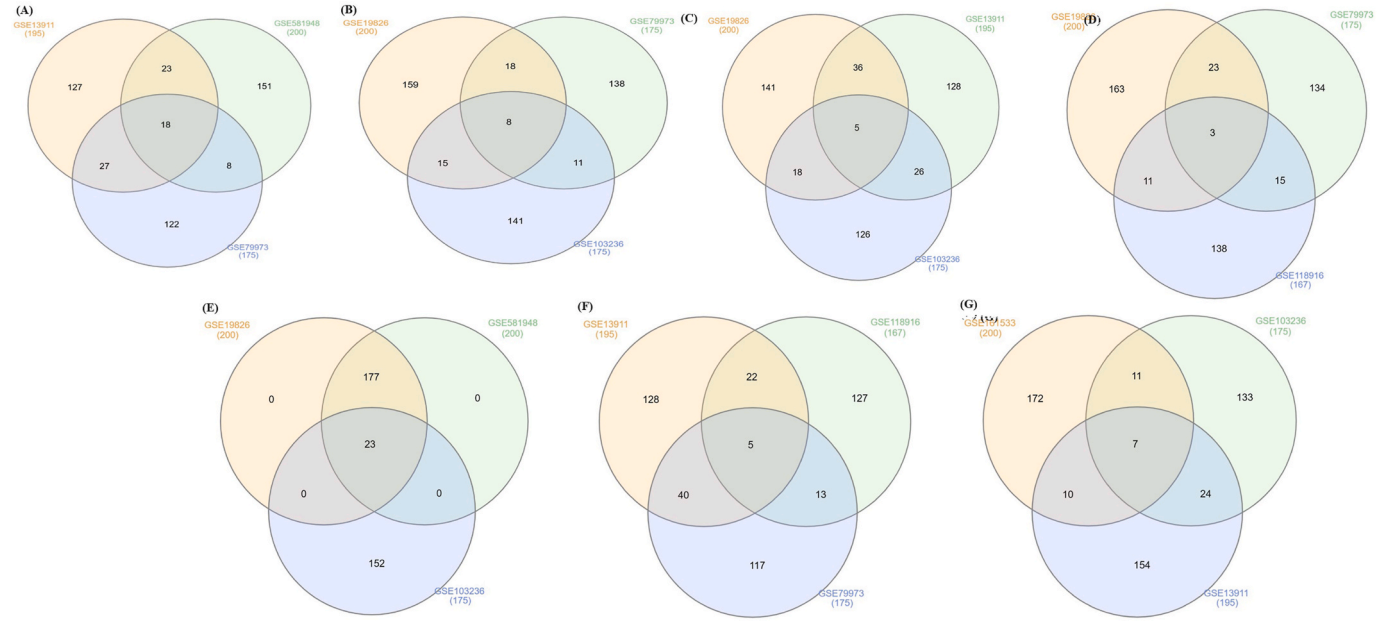

Fig. 3. Identification of DEG among the group of three dataset combinations. (A) DEGs analysis among GSE13911, GSE581948, and GSE79973 datasets combination. The 18 genes were noted to be common **(B)** DEGs analysis among GSE19826, GSE79973, and GSE103236 datasets combination. Only 8 genes were noted to be common (C) DEGs analysis among GSE19826, GSE13911, and GSE103236 datasets combination. Only 5 genes were noted to be common (D) DEGs analysis among GSE19826, GSE79973, and GSE118916 datasets combination. Only 3 genes were noted to be common **(E)** DEGs analysis among GSE19826, GSE581948, and GSE103236 datasets combination. The 23 genes were noted to be common (F) DEGs analysis among GSE13911, GSE118916, and GSE79973 datasets combination. Only 5 genes were noted to be common (G) DEGs analysis among the GSE161533, GSE103236, and GSE13911 datasets combination. Only 7 genes were noted to be common.

Table 1
KEGG pathway analysis of common DEGs among 05 datasets combinations.

Sr No.	Datasets Combination	Gene Count	Gene	Pathway
1.	GSE161533, GSE54129, GSE33651, GSE79973, GSE19826	1	ESSRG	Not predicted

Table 2
KEGG pathway analysis of 04 datasets combinations.

Sr No.	Datasets combinations	Gene count	Gene	Pathways
1.	GSE118916, GSE79973, GSE103236, GSE13911	1	ESRRG	Not predicted
2.	GSE33651, GSE79973, GSE118916, GSE161533	1	RAB31	Not predicted

and KCNJ16 across various TCGA cancer types was performed using UALCAN. The expression levels, presented as log2 (TPM + 1) values, reveal significant differences between tumor and normal samples (Fig. 6). ATP4B shows significant overexpression only in Lung adenocarcinoma (LUAD). ATP4A exhibits significant high expression in Bladder Urothelial Carcinoma (BLCA), Kidney chromophobe (KICH), Lung squamous cell carcinoma (LUSC), and Uterine corpus endometrial carcinoma (UCEC).

CCKBR demonstrates downregulation in all cancers, significant low expression was observed in Bladder Urothelial Carcinoma (BLCA), Breast Invasive Carcinoma (BRCA), Esophageal Carcinoma (ESCA), Glioblastoma Multiforme (GBM), Kidney Renal Clear Cell Carcinoma (KIRC), Kidney Renal Papillary Cell Carcinoma (KIRP), Liver Hepatocellular Carcinoma (LIHC), LUAD, LUSC, Prostate Adenocarcinoma (PRAD), stomach adenocarcinoma (STAD) and UCEC.

KCNJ15 displays significant overexpression in multiple cancers, including BLCA, colon adenocarcinoma (COAD), GBM, UCEC, Head and

Table 3
KEGG pathway analysis of 03 datasets combinations.

Sr. no.	Datasets combinations	Gene count	Genes	Pathways
1.	GSE 13911, GSE581948, GSE79973	18	CWH43, ATP4B, ATP4A, TRIM50, ESRRG, KCNJ16, CCKBR, GHRL, ADH7, AQP4, LINC00982, COL6A3, COL12A1, FGD4, DGKD, SPARC, CKMT2, KCNJ15,	Gastric acid secretion. (ATP4B, ATP4A, CCKBR, KCNJ15, KCNJ16) Collecting duct acid secretion. (ATP4B, ATP4A)
2.	GSE19826, GSE79973, GSE103236	8	ESRRG, CCKBR, CKMT2, APOBEC2, THY1, BGN, TIMP1, SPARC	
3.	GSE19826, GSE13911, GSE103236	5	ESRRG, CCKBR, CKMT2, ATP11A, SPARC	
4.	GSE19826, GSE79973, GSE118916	3	ESRRG, TMEM161B, ADH7	
5.	GSE19826, GSE581948, GSE103236	23	ESRRG, CCKBR, SMIM11A, DUSP19, CKMT2, GPER1, APOBEC2, CNTN3, THY1 ADHFE1, S100A10, CLDN7, ATP11A, BGN, TIMP1, PEBP4, CKB, SCUBE2, SPARC, TTYH3, ITIH5, WNT5A, PNPLA7	
6.	GSE13911, GSE118916, GSE79973	5	ESRRG, ADH7, CAPN13, SMIM6, PBLD	
7.	GSE161533, GSE103236, GSE13911	7	MYOC, SCARA5, PLCXD3, CKS1B, ATAD2, CKMT2, TNFRSF10B	

Table 4
Expression analysis of hub genes.

Sr.No.	Datasets	Genes				
		ATP4B	ATP4A	CCKBR	KCNJ15	KCNJ16
1	GSE31811	7.21	1.63	6.40	1.03	1.40
2	GSE26899	0.29	5.60	2.01	1.11	1.45
3	GSE26272	0.255	0.471	0.830	0.369	0.914

Neck squamous cell carcinoma (HNSC) and rectum adenocarcinoma (READ). Whereas, significant downregulation was noted in KICH, KIRC, KIRP, LIHC, LUAD, LUSC, prostate adenocarcinoma (PRAD), and STAD.

Lastly, except GBM, and Cholangiocarcinoma (CHOL), KCNJ16 expression was found to be significantly downregulated in BRCA, COAD, Cervical squamous cell carcinoma (CESC), ESCA, HNSC, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, and STAD (Fig. 6).

4. Discussion

Gastric cancer (GC) is one of the most lethal and prevalent cancers worldwide with a survival rate of less than 5 years [13]. Early diagnosis and proper treatment strategies can help reduce death due to GC [13]. The identification of genome-based biomarkers can improve the diagnosis, prognosis, and drug effectiveness. Therefore, the present study was conducted to investigate new potential diagnostic and prognostic biomarkers of GC. For this purpose, *in silico* microarray data sets analysis was performed to identify GC biomarkers. From the GEO datasets database, ten microarray gene expression datasets were obtained, and validation of the gene expression of identified DEGs was performed by three more datasets. The combinatorial analysis of studied datasets for the detection of shared DEG showed ESSRG and RAB31 genes to be commonly present in groups of 5 and 4 datasets. The DEG analysis of three-three dataset combinations discloses a total of 69 shared DEG (CWH43, ATP4B, ATP4A, TRIM50, ESRRG, KCNJ16, CCKBR, GHRL, ADH7, AQP4, LINC00982, COL6A3, COL12A1, FGD4, DGKD, SPARC, CKMT2, KCNJ15, ESRRG, CCKBR, CKMT2, APOBEC2, THY1, BGN, TIMP1, SPARC, ESRRG, CCKBR, CKMT2, ATP11A, SPARC, ESRRG, TMEM161B, ADH7, ESRRG, CCKBR, SMIM11A, DUSP19, CKMT2, GPER1, APOBEC2, CNTN3, THY1, ADHFE1, S100A10, CLDN7, ATP11A, BGN, TIMP1, PEBP4, CKB, SCUBE2, SPARC, TTYH3, ITIH5, WNT5A, PNPLA7, ESRRG, ADH7, CAPN13, SMIM6, PBLD, MYOC, SCARA5, PLCXD3, CKS1B, ATAD2, CKMT2, and TNFRSF10B) in combinations of different datasets.

In the presented study, 5 genes, namely ATP4B, ATP4A, CCKBR, KCNJ15, and KCNJ16, were identified to be common differentially expressed in GC patients.

Hydrogen/potassium ATPase A and B (ATP4A and ATP4B) are proton pump genes that play a vital role in gastric acid secretion [25]. The KEGG pathway analysis shows these genes to be involved in the gastric acid secretion pathway and collecting duct acid secretion pathways. The gastric H+/K + -ATPase pathway once activated causes the release of gastrin which regulates gastric acid production [26], growth of the gastrointestinal tract, and gastric acid secretion [27]. Abnormal gastrin and gastric acid secretion are known to lead to gastric carcinomas [28]. The H+/K + -ATPase is a heterodimeric P-type ATPase, comprised of two subunits, in the parietal cells involved in H+/K+ exchange using ATP. It is the essential unit of the ion transport pathway and regulates gastric acid secretion [29]. ATP4A is the α subunit of gastric H+/K+-ATPase enzymes located at the membrane of parietal cells of gastric. It has catalytic activity for the hydrolysis of ATP [25] and has multiple functional sites including ion recognition, acyl-phosphorylation, ATP-binding, and inhibitor-binding sites [30]. ATP4B is the β subunit of H+/K + ATPase and is responsible for the stabilization of catalytic α-subunit and also regulates the acid secretion

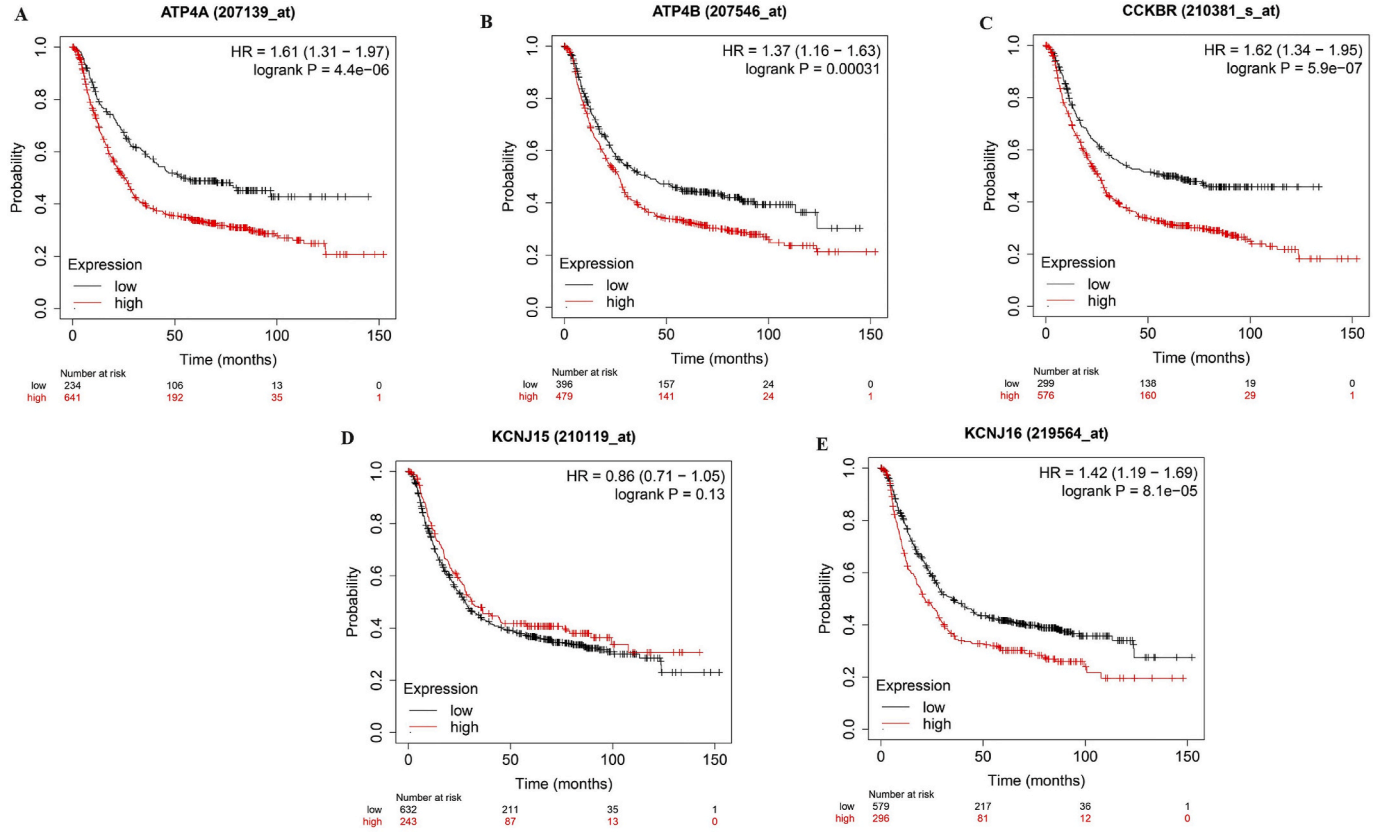

Fig. 4. Overall survival (OS) analysis of DEGs via Kaplan Meier Plotter. (A) OS analysis of ATP4A (B) OS analysis of ATP4B (C) OS analysis of CCKBR (D) OS analysis of KCNJ15 (E) OS analysis of KCNJ16. Logrank $p < 0.05$ was considered to be significant.HR=Hazard ratio.

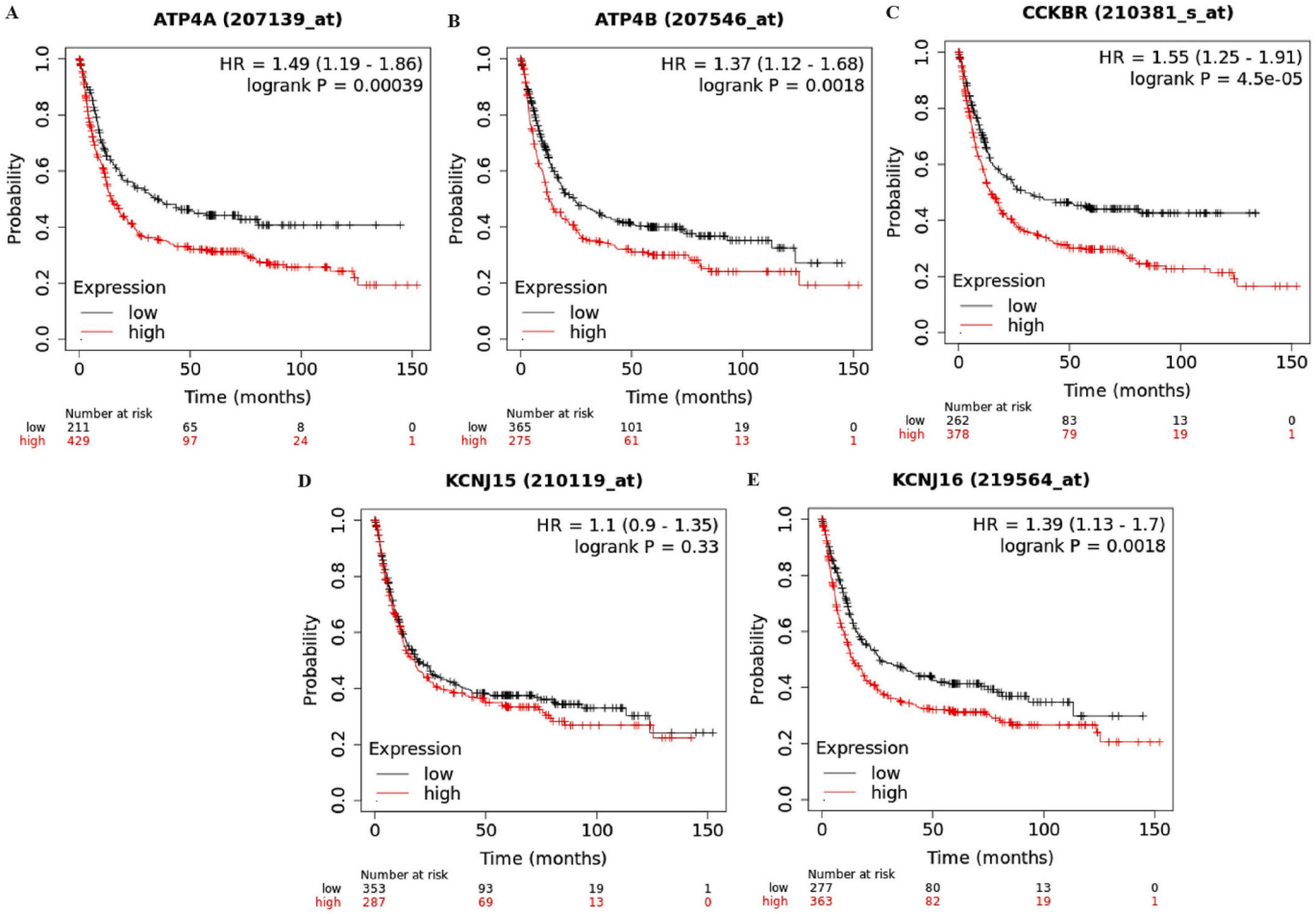

Fig. 5. Disease-free survival (DFS) analysis of DEGs via Kaplan Meier Plotter. (A) DFS analysis of ATP4A (B) DFS analysis of ATP4B (C) DFS analysis of CCKBR (D) DFS analysis of KCNJ15 (E) DFS analysis of KCNJ16. Logrank $p < 0.05$ was considered to be significant. HR=Hazard ratio.

pathway [25]. ATP4A and ATP4B normal expression is necessary for the integrity of gastric cell membranes and cellular differentiation [29]. The high H+/K + -ATPase activity, due to bacterial infection or inflammatory factors, resulted in excessive secretion of gastric acid [30]. High gastric acid secretion association with gastric ulcer is widely known. Nevertheless, the low gastric acid level was also detected to be carcinogenic [31]. In our study, both ATP4A and ATP4B were detected to be differently expressed. The analysis of ATP4A and ATP4B expression levels' impact on the survival of gastric cancer patients revealed poor survival in gastric cancer patients. Based upon these findings it is suggested that ATP4A and ATP4B aberrant expression might play a role in the worsening of gastric cancer and thus can be employed as prognostic biomarkers of gastric cancer to monitor overall and disease-free survival. Our study results are relevant to previous study reporting the downregulation of ATP4A in gastric carcinoma [32]. An earlier micro-array dataset-based study on gastric cancer identified ATP4A and ATP4B as downregulated DEGs which relate to poor overall survival in gastric cancer patients [33]. Another study on GEO datasets also reported the low expression of ATP4A in gastric carcinoma which was negatively associated with overall survival [34]. Previously *in silico* study also narrated the downregulation of ATP4A and ATPA4B suggesting them as diagnostic biomarkers of GC [35].

In the previous literature, gastric acid secretion was reported to be negatively associated with GC patient's age which implies that at the early stage of GC, gastric acid secretion will be low which increases with time at differentiated adenocarcinoma stage [36]. Whereas, gastrin was found to be positively linked with age showing a high level at the early stage of GC [36]. ATP4B also acts as a tumor suppressor gene (TSG) and was observed to be down regulated in GC patients as referred to above. Its downregulation was noted to be associated with the transformation of malignant gastric lesions [25]. Methylation was observed as one of the mechanisms of ATP4A and ATP4B downregulation in GC patients relative to the control [29]. Demethylation of ATP4A and ATP4B results in the activation of these genes and can inhibit GC progression thus confirming their role as TSG [35]. Restoration of ATP4B expression increased the inhibition of gastric cancer cell growth by chemotherapeutic drugs like docetaxel [37].

Similar to downregulated expression of ATP4A and ATP4B, their upregulation also contributes to gastric carcinoma. Gastric acid inhibitors like PPIs are in practice to reduce gastric acid secretion [28] by inhibiting proton pumps [26]. Thus, these proton pumps (highly active) are now considered as therapeutic targets and inhibiting gastric acid secretion using proton pump inhibitors (PPIs) is an effective strategy for treating gastric cancer [38].

These findings suggest that ATP4A and ATP4B both have an essential role in maintaining cell growth, and any dysregulation in their expression results in abnormal H+/K + -ATPase pathways and leads to gastric cancer progression. Monitoring ATP4A and ATP4B expression can help to predict the survival of patients and thus can be used as a drug target. Thus, ATP4A and ATP4B can be referred as prognostic and therapeutic targets of gastric cancer.

Furthermore, the pan-cancer analysis across TCGA cancer also represents significant downregulation of ATP4A in many cancers including Stomach adenocarcinoma (STAD), thymoma (THYM), thyroid

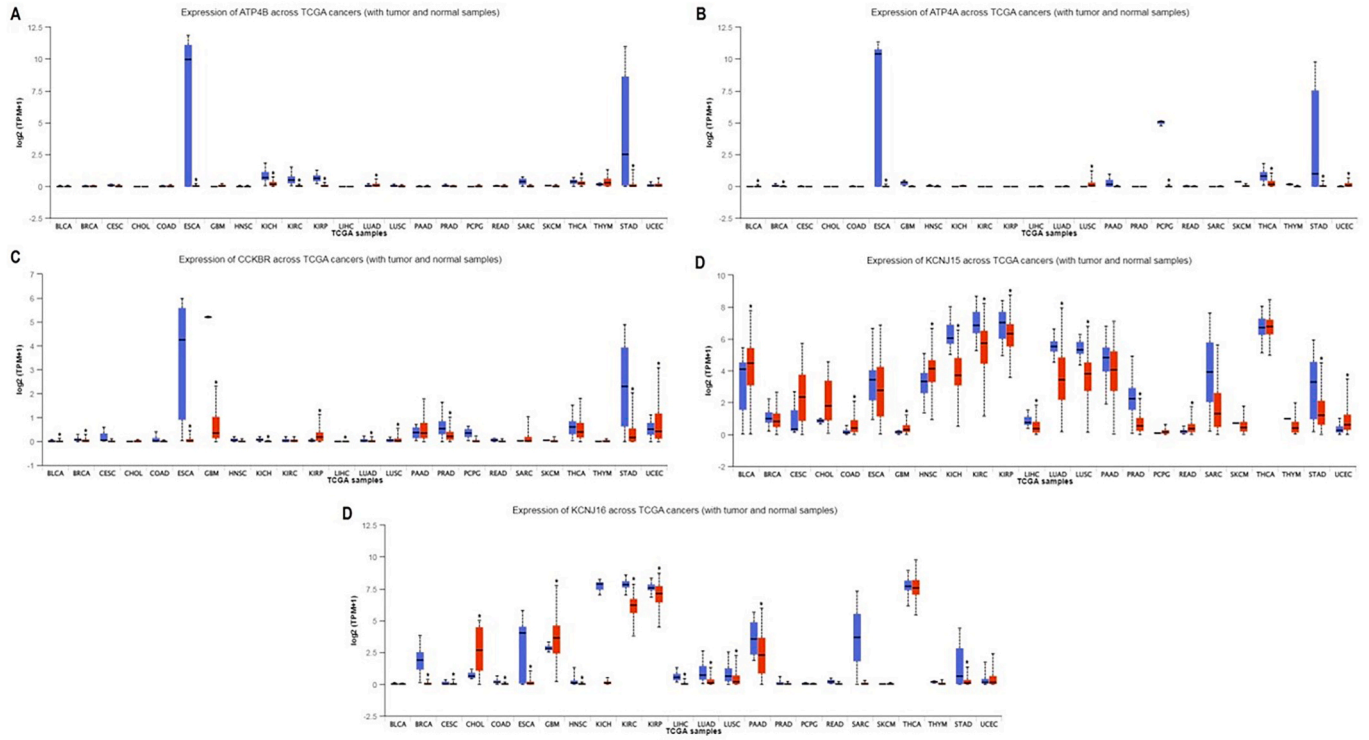

Fig. 6. Pan-cancer expression profiles of ATP4B, ATP4A, CCKBR, KCNJ15, and KCNJ16 across various TCGA cancer types. (A) ATP4B, (B) ATP4A, (C) CCKBR, (D) KCNJ15, and (E) KCNJ16 across different TCGA cancers are displayed. The log2 (TPM + 1) values are shown for tumor (red) and normal (blue) samples. Significant overexpression in specific cancers, indicated by asterisks, suggests the potential of these genes as pan-cancer biomarkers. P*-value <0.05. BLCA = Bladder Urothelial Carcinoma, BRCA = Breast Invasive Carcinoma, CESC = Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma, CHOL = Cholangiocarcinoma, COAD = Colon Adenocarcinoma, ESCA = Esophageal Carcinoma, GBM = Glioblastoma Multiforme, HNSC = Head and Neck Squamous Cell Carcinoma, KICH = Kidney Chromophobe, KIRC = Kidney Renal Clear Cell Carcinoma, KIRP = Kidney Renal Papillary Cell Carcinoma, LIHC = Liver Hepatocellular Carcinoma, LUAD = Lung Adenocarcinoma, LUSC = Lung Squamous Cell Carcinoma, PAAD = Pancreatic Adenocarcinoma, PCPG = Pheochromocytoma and Paraganglioma, PRAD = Prostate Adenocarcinoma, READ = Rectum Adenocarcinoma, SARC = Sarcoma, SKCM = Skin Cutaneous Melanoma, THCA = Thyroid Carcinoma, THYM = Thymoma, STAD = Stomach Adenocarcinoma, UCEC = Uterine Corpus Endometrial Carcinoma. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

carcinoma (THCA), Rectum adenocarcinoma (READ), Liver hepatocellular carcinoma (LIHC), Esophageal carcinoma (ESCA) and Breast invasive carcinoma (BRCA) whereas ATP4B was downregulated in STAD, KIRP, THCA, KIRC, KICH, ESCA, and BRCA. These findings are similar to previously reported studies where ATP4A and ATP4B expression was also observed to be downregulated in intestinal gastric cancer patients and the low expression resulted in the proliferation of epithelial cells [39]. Whereas, in lung cancer ATP4A and ATP4B were identified as upregulated DEGS among the 24 upregulated genes of mitochondrial energy metabolism pathway (MMRGs) which is the hallmark of lung cancer but no association with prognosis was observed [40]. A former pan-cancer study conducted on different cancer datasets i.e., colon, gastric, pancreatic, and ovary cancer also revealed significant downregulation of ATP4A and ATP4B only in gastric cancer and recommended ATP4A and ATP4B as diagnostic biomarkers of gastric cancer [41]. Moreover, another study on TCGA datasets identified ATP4A and ATP4B to be differentially expressed in esophageal carcinoma and further validated the downregulation of ATP4B by immunohistochemistry thus approving ATP4B as a diagnostic biomarker of esophageal carcinoma and validated *in silico* studies results [42]. The pan-cancer expression analysis suggested ATP4A and ATP4B as broad-spectrum diagnostic and prognostic biomarkers.

Cholecystokinin B-receptors (CCKBR) are the G-protein coupled receptors known to be expressed in the gastric mucosa enterochromaffin-like (ECL) cells i.e., NET type I I [43]. CCKBR normal signaling is regulated by the activation of phospholipase C-β/diacylglycerol/$Ca^{2+/}$ protein kinase C. For the gastric epithelium growth, gastrin is required [44]. CCKBR is the receptor of gastrin that stimulates acid secretion and gastrointestinal tract growth by binding to CCKBR [43]. CCKBR overexpression by activation through gastrin resulted in gastric adenocarcinoma development [45]. Epithelial cells expressing CCBR also secrete de novo gastrin, which also promotes gastric cancer cell growth and metastasis by autocrine mechanism [44].

In the current study, CCKBR was found to be DEG in gastric cancer. Pathway enrichment study represents its role in the gastric acid secretion pathway. The survival plots indicated an association of high CCKBR expression with reduced survival of gastric cancer patients.

Our results were in contrast to bioinformatic-based analysis on gastric cancer which identified 476 DEGs and 59 hub genes including CCKBR in gastric cancer but reported no significant association of CCKBR with gastric cancer prognosis [46]. Downregulation of CCKBR has a known impact on gastric cancer cell proliferation and death [47]. Its association with the worst survival of patients suggests its role in cancer progression and as a therapeutic target. Previously, it was observed that inhibition of CCKBR by netazepide decreased tumor size validating its role in cancer progression [43]. The relation of high CCKBR expression and poor patient survival, identified in the present study, suggests that CCKBR plays an important role in gastric cancer progression and thus can be considered as a diagnostic, prognostic, and therapeutic target. Its high expression resulted in the increased growth of gastric epithelial cells and high production of gastric acid thus leading to gastric ulcer and malignancy.

CCKBR was found to be expressed in all tissues but its high expression was not noted in all cancers [48]. Similar situations were unveiled by TCGA pan-cancer analysis where downregulation of CCKBR expression in multiple cancers was noted reinforcing the potential role of

CCKBR as a pan-cancer biomarker. These results were comparable to previous studies i.e., an earlier study highlighted the downregulation of CCKBR in esophageal carcinoma, and its expression was observed to be negatively associated with the development and occurrence of esophageal cancer [42]. In pancreatic cancer, downregulation of CCKBR was observed which halts the cell cycle in the G1 phase and thus inhibits tumor cells' proliferation. Further low CCKBR expression enhanced the activity of caspase-3, and TUNEL-positive cells, and lowered the expression of apoptotic proteins' inhibitors indicating its apoptotic role in pancreatic cancer [49]. Likewise, its downregulation was found in ER^+ - breast cancer patients [48]. The low expression of CCKBR shows a negative association with cancer progression. In contrast in pancreatic cancer, CCKBR high expression was noted to be involved in the progression of pancreatic cancer cells and was declared as a therapeutic target of pancreatic adenocarcinoma [50]. A former study shows CCKBR expression association with different stages of colon cancer as high CCKBR activity was involved in the development of colorectal cancer [51]. These results indicate the association of CCKBR with gastric cancer along with colon and pancreatic cancer and thus CCKBR can be employed as a diagnostic, prognostic, and therapeutic biomarker of gastric cancer.

KCNJ15 is also called IRKK code for potassium (K) channel and is normally expressed in the kidney, pancreas, and lungs [52] playing an essential role in the maintenance of the resting membrane potential of β cells of the pancreas and the negative regulation of insulin [53]. In our study, KCNJ15 was detected to be DEG in gastric cancer. The pathway enrichment analysis showed its role in the gastric acid secretion pathway. KCNJ15 role in gastric acid secretion via histamine stimulation is known [54] but no previous study reported its role in gastric cancer. Pan-cancer analysis shows dysregulated expression in different cancers. Its downregulation was observed in KICH, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, and STAD among the TCGA cancer types representing it as a wide-spectrum biomarker. It was previously noted to be downregulated in pancreatic ductal adenocarcinoma (PDAC) [55]. The survival analysis shows the negative association of KCNJ15 expression with overall and disease-free survival of gastric cancer patients.

In renal cell carcinoma (RCC), an association of downregulated expression of KCNJ15 with poor overall survival of clear cell renal cell carcinoma (ccRCC) patients was reported thus declaring KCNJ15 a prognostic biomarker of ccRCC. However, it acts as a tumor suppressor gene and overexpression of KCNJ15 leads to inhibition of RCC invasion, arrests cell cycle, and halts cell proliferation via MMP downregulation and p21 upregulation and thus can be considered as therapeutic target for RCC [56].

KCNJ15 was also detected as a significant prognostic biomarker of LUAD [57]. In ESCA, heightened expression of KCNJ15 has been correlated with aggressive tumor behavior and poor prognosis, suggesting that it may play a critical role in esophageal cancer progression [58]. In hepatocellular carcinoma (HCC), the KCNJ15 was known to be involved in cancer recurrence and thus considered as a drug target for HCC treatment to improve overall survival [59]. This analysis shows that KCNJ15 has a role in maintaining cell proliferation, and gastric acid secretion, and therefore any dysregulation in its expression leads to carcinogenesis. However, it was observed that KCNJ15 is not a specific biomarker for gastric cancer. It is a pan-cancer biomarker as various studies reported its role in multiple cancer development besides gastric cancer. It is the first study reporting its dysregulation in gastric cancer.

KCNJ16, a member of the inwardly rectifying potassium channel family, was identified to be differentially expressed in gastric cancer. KCNJ16 plays a role in the maintenance of cell structure and membrane transport. It is vital for epithelial cell differentiation, proliferation, cell adhesion, and epithelial-to-mesenchymal transition (EMT) [60]. KCNJ16-regulated pathway has been linked to ion transport and cellular homeostasis, which may contribute to cell proliferation and tumorigenesis [61]. The KEGG pathway analysis shows that it is involved in the gastric acid secretion pathway. However, its role in GC has not been explored in any previously published study but the pan-cancer analysis of TCGA datasets revealed a change in its expression in various cancers. The survival curve results represented an association of high KCNJ16 expression with poor survival of GC patients.

The downregulation of KCNJ16 was referred to as a prognostic biomarker of hepatocellular carcinoma (HCC) by an earlier bioinformatic study [62]. Formerly, dysregulation of KCNJ16 in various cancers was identified by pan-cancer analysis [63] which supports our pan-cancer results. Evaluation of transcriptomic datasets of TCGA and GEO databases shows KCNJ16 to be downregulated in thyroid cancer patients and further experimental analysis validated its low expression in thyroid cancerous tissues [60]. It was observed to be downregulated among the identified DEGs in pancreatic cancer but no prognostic potential was seen in pancreatic cancer [64]. Another study exploring the signaling pathways in bladder cancer reported KCNJ16 as a target of miR-1 but no association with bladder cancer has been explored [61]. The overexpression of KCNJ16 was found in collecting duct and renal tubules which may affect the tumor microenvironment by altering pH levels and ion concentrations, creating conditions that support cancer cell survival and proliferation. In ccRCC, KCNJ16's elevated expression is thought to play a role in renal cancer development by affecting potassium ion homeostasis and cell membrane potential [65].

These studies suggested that KCNJ16 plays a role in cell differentiation, proliferation, and EMT and its high expression promotes abnormal cell proliferation thus it can be considered a modulator of gastric cancer. Keeping in view its effect on poor survival of gastric cancer patients, KCNJ16 can be employed as a prognostic biomarker. It can also be explored as a potential therapeutic biomarker of gastric cancer and lowering its expression can enhance the survival rate. Furthermore, it can be regarded as a pan-cancer biomarker due to its expression dysregulation in other cancers.

4.1. *Challenges and future prospects*

The present study proposed a biomarker panel of 5 genes for gastric cancer patients but the clinical implementation of these biomarkers may face some challenges.

i. The major challenge is the validation of biomarker efficacy in Pakistani gastric cancer patients by experimental analysis. Although the identified genes serve as prognostic and therapeutic biomarkers globally, however, population heterogeneity and sample size can affect the biomarker effectiveness in Pakistani populations. Therefore, there is a need to first validate the predicted biomarkers by experimental and clinical testing on Pakistani gastric cancer patients to ensure their potential as biomarkers in the Pakistani population.
ii. The cancer heterogeneity is another problem for biomarker validation. Therefore, the validation of identified biomarkers via molecular analysis should be conducted on gastric cancer patients of different sub-types.
iii. Further, the validation of the prognostic and therapeutic potential of these biomarkers is necessary. For which *in vitro* and *in vivo* studies should be performed. The validation studies will help to design personalized drugs and enhance the survival rate of gastric cancer patients.

5. Conclusion

In conclusion, the present study used bioinformatics tools to analyze microarray-based gene expression datasets and explore biological processes and signaling pathways closely associated with gastric cancer occurrence and development. This study revealed a few important genes (ATP4B, ATP4A, CCKBR, KCNJ15, and KCNJ16) as promising diagnostic and prognostic biomarkers for gastric cancer patients. Furthermore, pan-cancer analysis exhibited differential expression of the studied

genes in multiple cancers suggesting their potential as diagnostic and prognostic biomarkers at a wide spectrum along with gastric cancer. As these biomarkers have been identified internationally in different populations but not studied in the Pakistani population, thus experimental and clinical studies must be conducted to validate them as biomarkers in Pakistani populations. Furthermore, there is a need to investigate the therapeutic potential of these biomarkers. The information thus generated can help to devise better therapeutic strategies and increase the survival rate of cancer patients.

CRediT authorship contribution statement

Arbaz Akhtar: Writing – original draft, Investigation, Formal analysis. **Yasir Hameed:** Methodology, Data curation. **Samina Ejaz:** Validation, Supervision, Conceptualization. **Iqra Abdullah:** Writing – review & editing.

Ethical statement

The study was approved by the Departmental Board of Study of Department of Biochemistry & Molecular Biology, The Islamia University of Bahawalpur.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101880.

Data availability

The analyzed GEO datasets GSE54129, GSE79973, GSE161533, GSE103236, GSE33651, GSE19826, GSE118916, GSE112369, GSE13911, and GSE81948 are available at (https://www.ncbi.nlm.nih.gov/geo/).

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