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Hypothesis paper: GDF15 demonstrated promising potential in Cancer diagnosis and correlated with cardiac biomarkers

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Abstract

Background Cardiovascular toxicity represents a significant adverse consequence of cancer therapies, yet there remains a paucity of effective biomarkers for its timely monitoring and diagnosis. To give a first evidence able to elucidate the role of Growth Differentiation Factor 15 (GDF15) in the context of cancer diagnosis and its specific association with cardiac indicators in cancer patients, thereby testing its potential in predicting the risk of CTRCD (cancer therapy related cardiac dysfunction).

Methods Analysis of differentially expressed genes (DEGs), including GDF15, was performed by utilizing data from the public repositories of the Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO). Cardiomyopathy is the most common heart disease and its main clinical manifestations, such as heart failure and arrhythmia, are similar to those of CTRCD. Examination of GDF15 expression was conducted in various normal and cancerous tissues or sera, using available database and serum samples. The study further explored the correlation between GDF15 expression and the combined detection of cardiac troponin-T (c-TnT) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP), assessing the combined diagnostic utility of these markers in predicting risk of CTRCD through longitudinal electrocardiograms (ECG).

Results GDF15 emerged as a significant DEG in both cancer and cardiomyopathy disease models, demonstrating good diagnostic efficacy across multiple cancer types compared to healthy controls. GDF15 levels in cancer patients correlated with the established cardiac biomarkers c-TnT and NT-proBNP. Moreover, higher GDF15 levels correlated with an increased risk of ECG changes in the cancer cohort.

Conclusion GDF15 demonstrated promising diagnostic potential in cancer identification; higher GDF15, combined with elevated cardiac markers, may play a role in the monitoring and prediction of CTRCD risk.

Keywords GDF15, Cancer, Cardiovascular toxicity, CTRCD, Biomarker

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Introduction

Cancer and cardiovascular disease (CVD), share common risk factors such as obesity, smoking, and diabetes, and exhibit overlap in the signaling pathways that govern both normal cardiovascular physiology and tumor growth [1, 2]. The incidence of cardiovascular toxicity during or after cancer treatment has been on the rise, with heart failure (HF) being the most prevalent and severe cardiovascular complication associated with cancer therapy [3]. This trend may be attributed to improved survival rates among cancer patients, which has led to an increased prevalence of cardiomyopathy associated with aging and changes in immune function. Additionally, the cardiotoxic effects of specific cancer treatments (including chemotherapy, targeted therapy, biological agents, and irradiation) have become more pronounced [4]. Consequently, there is a pressing need to enhance the prevention, surveillance, and early management of cardiovascular diseases in patients who are at high risk of cardiac dysfunction related to cancer therapeutics throughout their treatment journey [5]. While cardiac biomarkers such as cardiac troponin-T (c-TnT) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) have been somewhat effective in guiding the initiation and monitoring of heart-protective therapy in cancer patients, there is a high demand for more sensitive and specific markers [6].

Growth Differentiation Factor 15 (GDF-15), a member of the transforming growth factor-beta (TGF-β) superfamily, is also known as macrophage inhibitory cytokine-1 (MIC-1) due to its role in inhibiting macrophage secretion of pro-inflammatory factors [7]. GDF15 is associated with a wide range of biological functions in both physiological and pathological processes, as evidenced by its alternative names [8]. Under normal conditions, GDF15 expression remains low in various tissues and serum but markedly increases in response to inflammation, tissue damage, and various disease states, including malignant tumors, CVD, diabetes, and obesity, thus acting as a stress response molecule [9–13]. As a diagnostic and prognostic marker for tumors, the expression of GDF15 correlates with the degree of cachexia [14]. Similarly, as a cardiovascular disease marker, it is closely related to heart failure and myocardial infarction severity [15, 16]. However, the potential of GDF15 as a predictive biomarker for CTRCD (cancer therapy related cardiac dysfunction) remains unclear, and its efficacy in assessing and monitoring cardiovascular toxicity during cancer treatment necessitates further experimental validation [17, 18].

In the current study, we identified cardiac biomarkers (including GDF15) in cancer patients using TCGA and GEO databases, and confirmed the efficiency of GDF15 in cancer identification by using serum samples, revealing the potential of GDF15 in the monitoring and predicting risk of CTRCD.

Materials and methods

Overall method framework

Recent cancer statistics indicate that approximately a quarter of all estimated cancer deaths can be attributed to digestive system tumors (DSTs) [19]. To identify cardiac biomarkers in cancer patients, we analyzed differentially expressed genes (DEGs) common to various DSTs using the TCGA database, supplemented by GEO database data related to cardiomyopathy (Figure S1). Among the five extracellular differential molecules identified across the two disease model datasets, GDF15 emerged as the most significant. Subsequent analysis revealed widespread expression of GDF15 across various normal and tumor tissues. Serum samples from 30 healthy donors and 507 cancer patients indicated that GDF15 is highly expressed in nearly all tumors, signifying significant diagnostic efficacy. Moreover, GDF15 serum levels showed a significant correlation with the cardiac markers NT-proBNP and c-TnT, particularly in cases involving acute heart failure and myocardial injury (Figure S2). An evaluation of electrocardiogram (ECG) results for cancer patients with varying GDF15 expression levels revealed a higher incidence of arrhythmic (e.g., sinus bradycardia, sinus tachycardia) and ischemic (e.g., ST changes, T-wave alterations) conditions among patients with elevated GDF15 levels, whereas patients with lower expression levels frequently exhibited normal ECG results.

Patients and healthy donors

This study enrolled a cohort comprising 30 healthy donors and 507 cancer patients treated at the Shandong Cancer Hospital and Institute from January 2022 to June 2023. The healthy donors consisted of individuals undergoing routine physical examinations, all of whom underwent serum GDF15 testing. Of these, five cases were also assessed for both c-TnT and NT-proBNP. The cancer patient group encompassed various malignancies, with 450 patients completing the full spectrum of tests for GDF15, cTnT, and NT-proBNP, and 57 liver cancer patients undergoing testing for GDF15 expression only. Comprehensive clinical data for the 450 patients across various tumor types (Table S1) were extracted from electronic medical records and the Ruimei Laboratory Information System version 6.0 (rmlis, Huangpu District, Shanghai, China), as summarized in Table 1. Informed consent was obtained from all participants prior to the study, with ethical approval granted by the Ethics Committee of the Shandong Cancer Hospital and Institute, aligning with the Declaration of Helsinki.

Table 1 The clinical characteristics of all samples

Characteristics	No. cases	Pro-BNP		c-TnT		GDF-15	
		Median	*P*-value	Median	*P*-value	Median	*P*-value
Normal	***5***/30[&]	***35.63***	*0.6028*	***3.12***	*0.3277*	760.50	*< 0.0001*
Cancer	***450***/507[#]	***321.45***		***10.94***		3109.80	
Age (year)							
≥ 59(59–88)	271(60%)	438.00	*0.0129*	11.54	*0.3762*	3197.01	*0.0008*
< 59(24–58)	179(40%)	144.99		10.02		2541.89	
Gender, n (%)							
Male	287(64%)	341.43	*0.6469*	12.26	*0.0369*	3071.45	*0.0571*
Female	163(36%)	286.26		8.61		2698.66	
Cancer type, n (%)							
Lung cancer	228	225.34	0.0159	9.90	0.0020	2522.94	< 0.0001
Liver cancer	***55***/112[#]	***546.33***		***8.58***		4161.95	
Esophageal cancer	46	205.06		11.13		2850.95	
Gastric cancer	39	631.49		13.73		4119.01	
Colorectal cancer	18	419.76		8.75		4413.80	
Breast	13	126.56		9.74		1710.49	
Lymphoma	22	90.88		8.45		2089.20	
Other*	**29**	620.90		23.35		3399.39	

& Normal control group consisted of 30 individuals, all of whom underwent serum GDF15 testing. Of these, five cases were also assessed for both c-TnT and pro-BNP

The cancer patient group encompassed 507 cases of various malignancies, with 450 patients completing the full spectrum of tests for GDF15, cTnT, and proBNP, and 57 liver cancer patients undergoing testing for GDF15 expression only

* Others include 2 cases of cervical cancer, 3 cases of endometrial cancer, 2 cases of ovarian cancer, 1 case of prostate cancer, 1 case of choriocarcinoma, 1 case of tongue cancer, 1 case of duodenal cancer, 1 case of pharyngeal cancer, 4 cases of pancreatic cancer, 5 cases of multiple myeloma, 1 case of melanoma, 2 cases of malignant pleural mesothelioma, 1 case of liposarcoma, 2 cases of brain tumor, 1 case of small cell lung cancer, 1 case of thymic cancer, a total of 29 cases

Colorectal cancer includes: colon cancer and rectal cancer

Lymphoma is a general term without specific classification, including B-cell lymphoma, Hodgkin's lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, etc

p value was calculated by Unpaired t test between two groups. Comparison among three groups was analyzed by Kruskal-Wallis test for abnormally distributed data. The numerical value was represented by Mean ± SD

Data collection

Data for this research were sourced from electronic medical records and the Ruimei Laboratory Information System. The c-TnT and NT-proBNP levels in cancer patients were determined using Electrochemiluminescence on the Cobas e801 analyzer (Roche Diagnostics, GmbH, Mannheim, Germany). The normal range for NT-proBNP was considered to be 0-125 pg/ml, with < 125 pg/ml exclusionary of chronic heart failure and < 300 pg/ml exclusionary of acute heart failure. For the diagnosis of acute heart failure using NT-proBNP levels, the criteria vary by age: for individuals younger than 50 years, a level greater than 450 pg/ml is indicative; for those aged between 50 and 75 years, the threshold is above 900 pg/ml; and for those over 75 years, a level exceeding 1800 pg/ml is suggestive of acute heart failure. Additionally, for patients with a glomerular filtration rate (GFR) below 60 ml/min, a NT-proBNP level greater than 1200 pg/ml is indicative. Regarding cardiac troponin-T (c-TnT), normal levels range from 0 to 14 pg/ml. Levels between 15 and 52 pg/ml suggest myocardial injury, while levels above 52 pg/ml are indicative of acute myocardial injury. The delineation of test range and criteria, which are diagnostic criteria based on many previous studies and the long-term data accumulation of *Roch* company in hospital detection operation [20, 21].

Enzyme-linked immunosorbent assay (ELISA)

Plasma samples were collected, centrifuged again at 3500 ×g for 10 min to eliminate hemocytes, including red blood cells, white blood cells, and platelets, and the supernatant was retained. The plasma levels of GDF15 from cancer patients and healthy donors were quantified using Human GDF-15 ELISA Kits (ab155432, Abcam, Cambridge, UK). Samples underwent a 10-fold dilution prior to testing, with subsequent procedures conducted as per the kit's protocol.

Database and processing

Analysis of differentially expressed genes (DEGs) in colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), stomach adenocarcinoma (STAD), lung adenocarcinoma (LUAD) tissues, and adjacent tissues was conducted using the GEPIA2 online database (http://gepia2.cancer-pku.cn/#dataset). Detailed clinical data of cancer patients were obtained from The Cancer Genome Atlas (TCGA) database.

Additionally, public gene expression profiles (GSE116250) covering 14 non-failing donors (NF), 37 dilated cardiomyopathy (DCM), and 13 ischemic cardiomyopathy (ICM) were examined. DEGs identification and visualization between NF, DCM and ICM were executed through volcano plots and heatmaps. Extracellular gene analysis for protein subcellular localization utilized Hum-mPLoc 3.0.

ECG analysis

ECG data, collected on the day of or within one day before or after blood sampling, were recorded with 12-lead ECG devices and interpreted by a minimum of two cardiologists. The analysis included normal ECG readings and identification of arrhythmic (e.g., sinus bradycardia, sinus tachycardia, incomplete right bundle branch block, intraventricular block), ischemic (e.g., ST changes, T-wave alterations), and non-specific (e.g., low-voltage QRS, QT interval variations) findings.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, CA, USA). Data were analyzed using the unpaired t-test for two groups with normal distribution, the Mann-Whitney test for non-normally distributed data, the Kruskal-Wallis test for multiple groups with non-normally distributed data, and one-way ANOVA for normally distributed datasets. Results are presented as mean±standard deviation (SD), with a p-value of <0.05 considered statistically significant.

Results

Screening of DEGs in TCGA database

Given the high incidence and mortality associated with digestive system tumors (DSTs), we sourced data for colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), liver hepatocellular carcinoma (LIHC), and stomach adenocarcinoma (STAD) from the TCGA database, comprising a total of 1234 cancerous and 1006 non-cancerous tissue samples. The data included colon adenocarcinoma (COAD; 275 cancer vs. 349 control tissues), esophageal carcinoma (ESCA; 182 cancer vs. 286 control tissues), liver hepatocellular carcinoma (LIHC; 369 cancer vs. 160 control tissues), and stomach adenocarcinoma (STAD; 408 cancer vs. 211 control tissues), as detailed in Table S2. DEGs were determined based on a fold change>2.0 and a P-value<0.05, and their distribution was illustrated in volcano plots (Fig. 1A-D, Tables S3-6). An intersection of DEGs across these DSTs revealed 381 common genes (Fig. 1E, Tables S7-10), with LIHC-specific DEGs showcased in a heatmap (Fig. 1F). Given the need for biomarkers detectable in serum, we conducted a sub-localization analysis using Hum-mPLoc 3.0, identifying 48 extracellularly localized DEGs (Tables S11-14), among which GDF15 was highlighted as a significant finding (Fig. 1G).

Identification of DEGs clusters attributed to cardiomyopathy and DSTs patients

Integrating cardiomyopathy data from GSE116250, including 14 non-failing donors (NF), 37 dilated cardiomyopathy (DCM), and 13 ischemic cardiomyopathy (ICM) cases, we screened for DEGs with fold changes>2.0 and $P<0.05$, as depicted in volcano maps (Fig. 2A-B, Tables S15-16). This analysis identified 629 DEGs common between the two cardiomyopathy types (Fig. 2C). A further screen of serum biomarkers using Hum-mPLoc 3.0 revealed 105 extracellular DEGs, as displayed in heatmaps (Fig. 2D-E, Tables S17-18). A Venn diagram pinpointed 19 DEGs shared between 4 types of DST and 2 types cardiomyopathy patients, with 5 extracellular molecules identified for potential clinical blood detection, most notably GDF15 (Fig. 3B-C, Tables S19-22). The differential expression of GDF15 in lung adenocarcinoma (LUAD)/adjacent tissues was not as pronounced as in DSTs (Fig. 3D).

GDF15 as a diagnostic biomarker for cancer patients

In pursuit of clinical insights on GDF15, we utilized integrated databases such as the Protein Atlas (https://www.proteinatlas.org/ENSG00000130513-GDF15) to examine its expression across 44 diverse tissues. This analysis, underpinned by knowledge-based annotations, utilized color-coding to demarcate tissue groups sharing functional similarities. Notably, GDF15 exhibited significant spatial expression specificity, prominently within most digestive system tissues, albeit with notable exceptions in the liver and esophagus. Immunohistochemical assays revealed distinct cytoplasmic staining patterns in colorectal and prostate cancers, among others, demonstrating the presence of GDF15. In contrast, lung cancer and certain other tumors displayed minimal to no GDF15 staining, a finding that aligns with previous analyses from the TCGA database (Fig. 4A-B). We utilized the proximity extension assay (PEA) to measure plasma concentrations of GDF15 across various cancer types. Notably, the serum levels of GDF15 in different cancer types did not always align with the patterns observed in our tissue staining (Fig. 4C). Serum samples from 30 healthy donors and 507 cancer patients representing a diverse array of cancers were analyzed to ascertain GDF15 concentrations (Table 1). In the healthy cohort ($N=30$), the mean GDF15 concentration was 760.5±60.30 pg/mL. In cancer patients ($N=507$), however, GDF15 levels were markedly elevated, with mean concentrations ranging from 1710±656.2 to 4162±214.8 pg/mL (Fig. 4D). Earlier observations had indicated that normal liver tissues

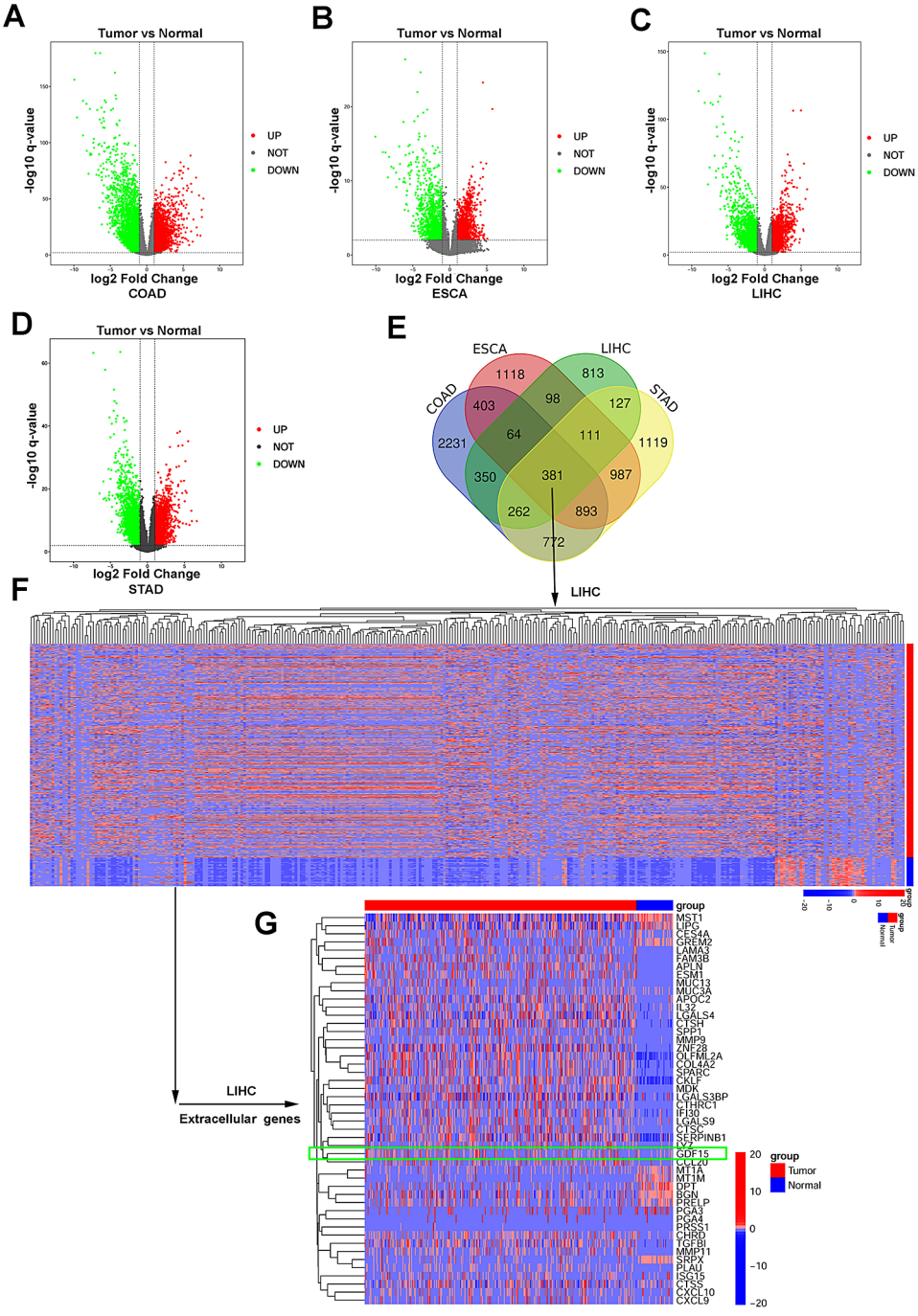

Fig. 1 DEGs identification in the TCGA database. Volcano plots comparing the expression fold-change of DEGs in COAD tissues **(A)**, ESCA tissues **(B)**, LIHC tissues **(C)**, STAD tissues **(D)** compared with adjacent normal tissues. **(E)** Venn plot showing the shared genes among the DEGs in 4 kinds of DST tissues vs. healthy control tissues, and displayed in a heatmap (**F**, up-regulated marked in red or down-regulated marked in blue). **(G)** Shared DEGs that extracellular localized were screened and presented by heatmap (LIHC vs. normal tissues), and GDF15 was among them

exhibited minimal or no GDF15 staining, whereas liver cancer tissues and serum samples displayed significantly higher GDF15 levels, the highest among all examined cancer types. Similarly, GDF15 staining was either weak or absent in both healthy and cancerous lung tissues, yet serum levels of GDF15 were considerably increased in lung cancer patients.

To evaluate the diagnostic utility of GDF15 for cancer, we compared serum GDF15 levels between representative cancer patient groups and healthy controls, employing receiver operating characteristic (ROC) curves.

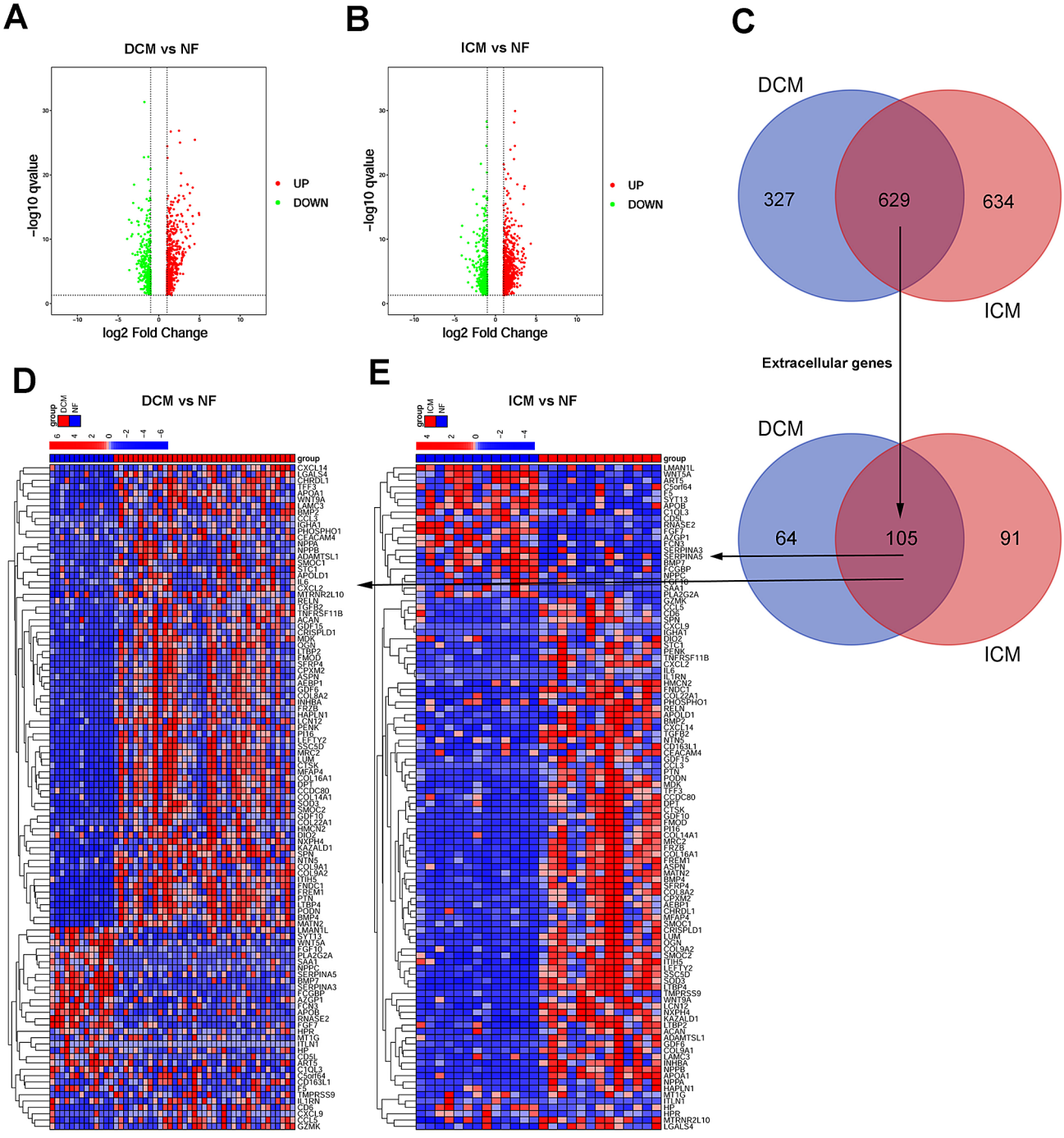

Fig. 2 Screening of DEG clusters attributed to cardiomyopathy. Volcano plots comparing the expression fold-change of DEGs in dilated cardiomyopathy (DCM) vs. non-failing donors (NF) **(A)**, and ischemic cardiomyopathy (ICM) vs. NF **(B)**. **(C)** Venn plot showing the shared genes among the DEGs in DCM and ICM vs. NF, and the shared extracellular localized DEGs, presented in heatmaps (**D**, **E**); these include GDF15

GDF15 demonstrated significant discriminative ability for pan-cancer detection, achieving an area under the curve (AUC) of 0.91 (95% confidence interval [CI] 0.8719–0.9396), with a sensitivity of 84.02% and a specificity of 86.67% compared to healthy controls (Fig. 5A). The diagnostic performance of GDF15 varied across the various cancers, exhibiting particularly strong diagnostic efficacy in liver cancer, with an AUC of 0.99 (95% confidence interval [CI] 0.9731–1.001), 92.86% sensitivity, and 96.67% specificity (Fig. 5B-F). However, in breast cancer, the diagnostic value of GDF15 was notably lower, evidenced by an AUC of 0.51 ($p=0.9578$), with 30.77% sensitivity and 96.67% specificity (Fig. 5E), possibly due to the

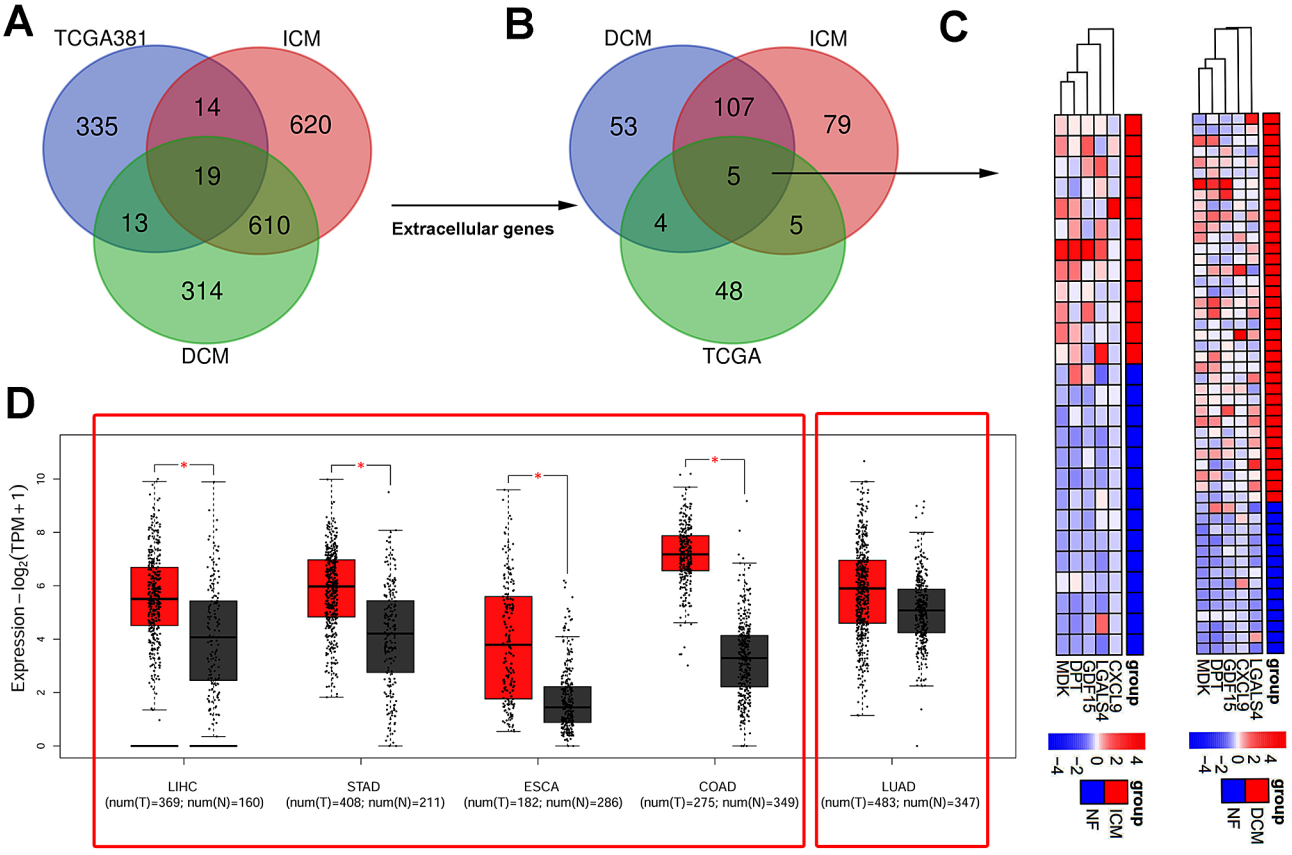

Fig. 3 Identification of DEG clusters attributed to cardiomyopathy and DST patients. Venn plot showing DEGs shared among DST patients **(A)**, from which five differential extracellular molecules were identified **(B)** and are depicted in a heatmap **(C)**. Additionally, panel **(D)** displays GDF15 expression levels in various tumor tissues compared to control tissues, as recorded in the TCGA database. Statistical data are expressed as mean ± standard deviation (SD), with significance determined by an unpaired two-tailed t-test: * indicates $p < 0.05$

limited number of breast cancer cases ($N=13$) included in the study.

Serum GDF15 levels in cancer patients correlated with cardiac indicators

Cancer therapy related cardiovascular toxicity includes myocardial injury and heart failure, immune myocarditis, hypertension, arrhythmia, coronary heart disease, venous thromboembolism, dyslipidemia. The correlation of these markers with cardiac dysfunction, as detailed in the *Methods and Materials* section, varies across the spectrum of potential values. Despite most c-TnT and NT-proBNP values falling within or near the normal range, with outliers presenting scattered results, it was challenging to deduce a straightforward relationship between GDF15 levels and these cardiac markers using a simple correlation analysis approach. Thus, we observed GDF15 expression across various degrees of cardiac disease in a pan-cancer context. We discovered that as c-TnT and NT-proBNP levels rose, indicating worsening cardiac disease, GDF15 expression also significantly increased (Fig. 6A-B). The mean concentration of GDF15 in individuals with NT-proBNP within the normal range (<125 pg/ml, $N=287$) was approximately 2507±110.4 pg/mL. This included individuals with NT-proBNP levels below 10 pg/ml ($N=35$), where GDF15 levels averaged about 1523±184.4 pg/mL. When NT-proBNP levels suggested chronic heart failure (>125 & <300 pg/ml, $N=88$), GDF15 concentrations averaged around 3044±226.0 pg/mL, $P=0.0230$. For NT-proBNP levels indicating potential acute heart failure (>450 pg/ml, $N=55$), GDF15 levels rose to an average of 4850±306.7 pg/mL, $P<0.0001$. Notably, in cases with NT-proBNP exceeding 1500 pg/ml ($N=17$), GDF15 levels reached a particularly high average of 5933±395.4 pg/mL, $P<0.0001$ (Fig. 6A).

When evaluating c-TnT levels, individuals within the normal range (<14 pg/ml, $N=366$) had a mean GDF15 concentration of 2615±101.1 pg/mL. This included a subset ($N=13$) with c-TnT levels below 3 pg/ml, where GDF15 averaged approximately 1283±277.5 pg/mL. For patients with c-TnT levels indicative of myocardial injury (15–52 pg/ml, $N=82$), GDF15 levels averaged around 4303±242.2 pg/mL, $P<0.0001$. In cases of acute myocardial injury (c-TnT>52 pg/ml, $N=4$), GDF15

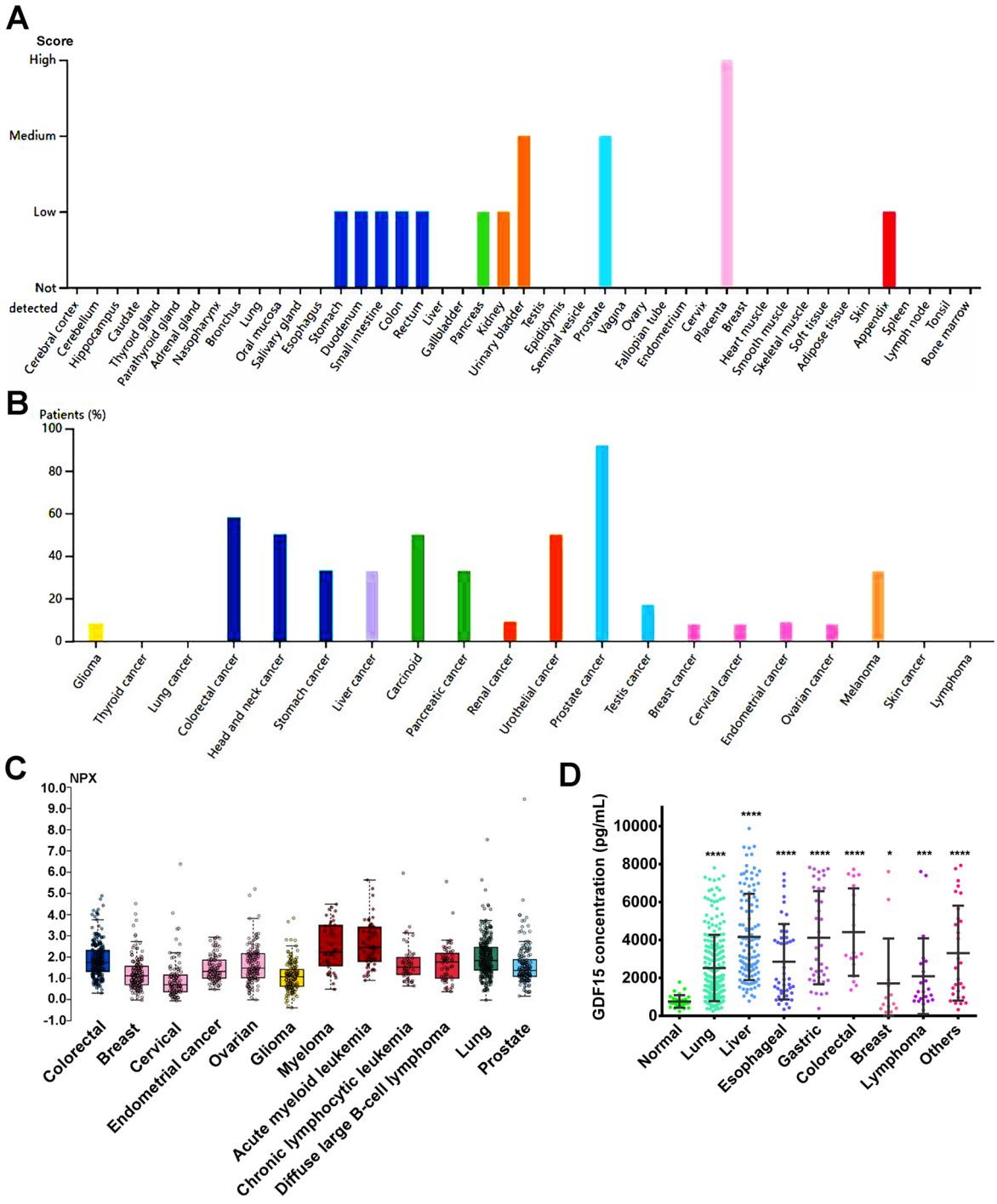

Fig. 4 Expression profile of GDF15 in human normal / tumor tissues and blood. The expression of GDF15 provided by available analysis platforms in normal human tissues **(A)** against tumor tissues **(B)**. Panel **(C)** shows the serum GDF15 levels in patients with various tumors, as reported in an online database, while panel **(D)** illustrates the concentrations of GDF15 that we determined in the serum of cancer patients in comparison to healthy controls. The data are expressed as mean ± standard deviation (SD), with statistical analysis conducted via an unpaired two-tailed t-test, where * signifies $p < 0.05$, *** signifies $p < 0.001$, and **** signifies $p < 0.0001$

concentrations escalated to an average of 5789 ± 1151 pg/mL, $P = 0.0012$ (Fig. 6B).

Further analysis was conducted on lung and liver cancer patients, representing the largest subsets in this study, to more closely explore the correlation between GDF15 expression and cardiac biomarkers. Given that most c-TnT or NT-proBNP values were within or near normal ranges, with a limited number of cases showing

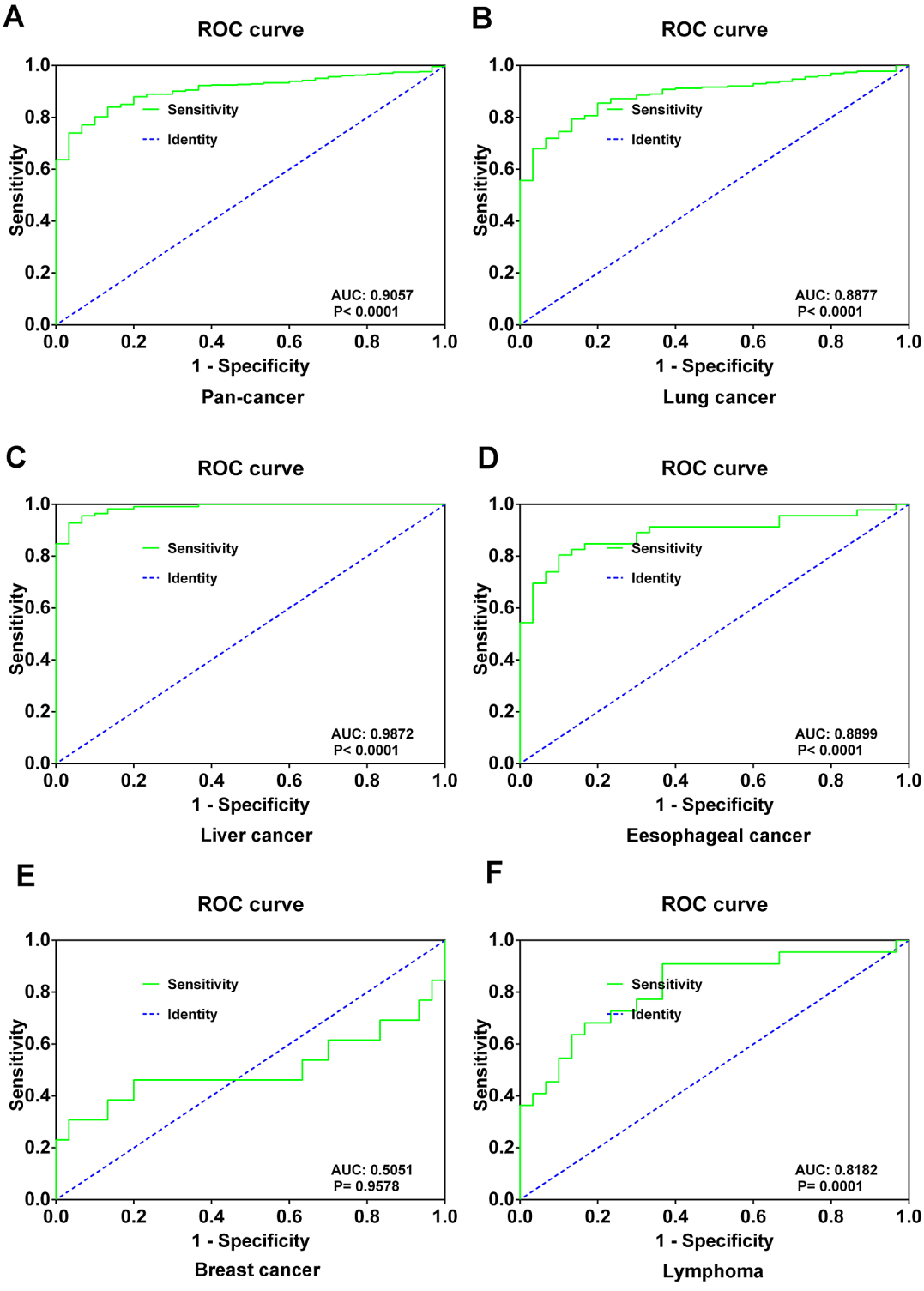

Fig. 5 Diagnostic utility of GDF15 in various cancers. **(A)** ROC curves revealed the AUC of pan-cancer to be 0.9057, $P < 0.0001$. ROC curves for GDF15 in the diagnosis of lung cancer **(B)**, liver cancer **(C)**, esophageal cancer **(D)**, breast cancer **(E)**, and lymphoma **(F)**

abnormal expression, the correlation findings for these specific cancer types were not as pronounced as those observed in the broader cancer cohort. GDF15 levels were only significantly correlated with acute heart failure (NT-proBNP > 450 pg/ml) in lung cancer patients (Fig. 6C-D). In instances of myocardial injury (c-TnT > 14 pg/ml), GDF15 expression markedly increased in both lung and liver cancer (Fig. 6E-F).

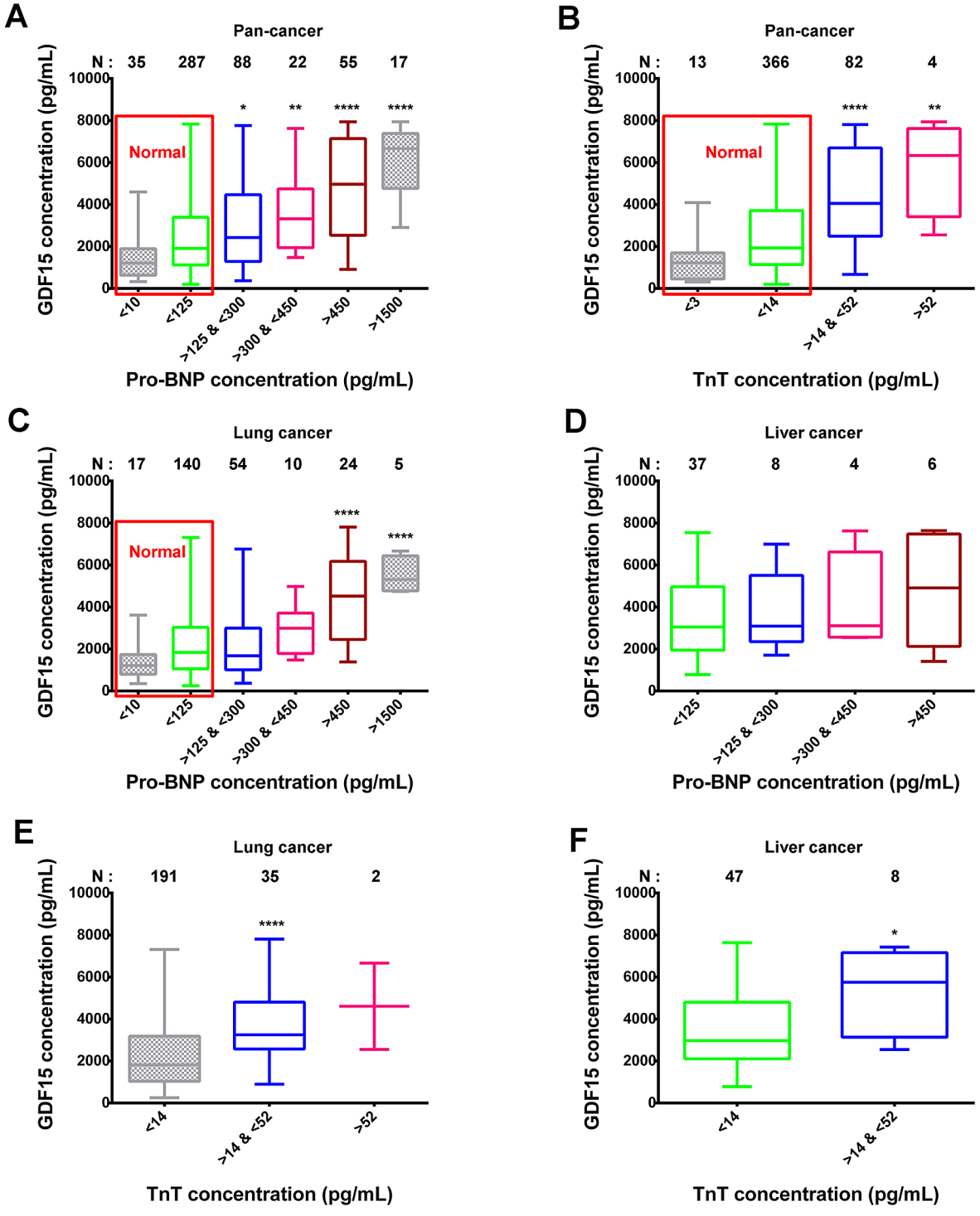

Fig. 6 Association Between Serum GDF15 Levels and Cardiac Biomarkers in Cancer Patients. This figure displays the correlation of serum GDF15 levels with cardiac biomarkers across all cancer types, with panels **(A)** and **(B)** illustrating GDF15 levels across varying NT-proBNP and c-TnT ranges, respectively. Panels **(C)** and **(D)** detail GDF15 levels in lung cancer patients across different NT-proBNP ranges, while panels **(E)** and **(F)** depict these levels in liver cancer patients across varying c-TnT ranges. Data represent mean ± standard error of the mean (SEM), with normal ranges for c-TnT and NT-proBNP serving as controls. N: represented the number of cases. Statistical significance was assessed using an unpaired two-tailed t-test, where * indicates $p < 0.05$, ** indicates $p < 0.01$, and **** indicates $P < 0.0001$

The levels of serum GDF15 associated with ECG changes

Electrocardiography (ECG) has historically been used for screening and monitoring of cardiovascular toxicity caused by cancer treatments [22], however it could not be completed on all patients at the times of blood collection due to various circumstances. For instance, the patient with the highest serum level of GDF15 (7493.66 pg/ml) died the day following blood collection, leaving no ECG data. Consequently, we selected a subset of 26 patients, characterized by either relatively high or low serum GDF15 levels, for analysis and comparison of ECG results, as summarized in Table 2. The ECG findings of six representative cases are illustrated in Fig. 7. This analysis revealed that patients with lower serum levels of GDF15 generally had low levels of c-TnT or NT-proBNP, correlating with predominantly normal ECG outcomes (Fig. 7A-B). Conversely, when serum GDF15 exceeded 600 pg/ml, ECGs exhibited sinus rhythm and ST changes, despite c-TnT and NT-proBNP values remaining within normal limits (Table 2). Moreover, patients presenting with higher GDF15 expression were found to have elevated levels of c-TnT or NT-proBNP, alongside arrhythmic alterations (such as sinus bradycardia and tachycardia) and significant ischemic changes (including ST changes and T-wave alterations), suggesting a notable correlation among these biomarkers (Fig. 7C-D; Table 2). One particular case involved a patient with a high GDF15 expression (6637.38 pg/ml) whose serum levels of c-TnT (6.84 pg/ml) and NT-proBNP (16.57 pg/ml) were within normal ranges, yet the ECG revealed ST depression in

Table 2 The clinical characteristics of representative patients with ECG outcomes

Cancer type	Age (year)	Sex	GDF-15 pg/ml	Pro-BNP[#] pg/ml	c-TnT[&] pg/ml	Description of ECG changes
Brain	24	F	328.47	**< 10.00**	**< 3.00**	Normal ECG
Lung	36	M	351.00	**< 10.00**	**6.48**	Normal ECG
Gastric	70	F	388.14	**68.78**	**3.31**	Normal ECG
Lung	59	F	566.89	**34.76**	**6.14**	Normal ECG
Lung	35	F	572.02	**47.52**	**3.04**	Normal ECG
Breast	35	F	604.52	**32.41**	**4.41**	Normal ECG
Lung	57	M	620.52	**< 10.00**	**6.03**	Sinus rhythm, ST change
Esophageal	40	F	631.81	**< 10.00**	**5.40**	Sinus rhythm, ST change
Liver	73	M	778.14	**12.76**	**7.34**	Sinus rhythm, T-wave changes
Esophageal	53	M	823.84	**18.25**	**4.76**	Normal ECG
Liver	63	M	1045.85	**35.28**	**5.53**	Sinus bradycardia
Lung	58	M	1383.57	**10.91**	**4.34**	Normal ECG
Lung	75	M	2044.60	553.50	**10.48**	QS pattern in leads V1-2, Sinus rhythm
Lung	37	M	6208.85	1561.00	49.85	Sinus tachycardia, Intraventricular block, Prolonged QT interval, Clockwise rotation
Lung +	63	M	6637.37	**16.57**	**6.84**	Sinus rhythm, Incomplete right bundle branch block, ST-depression in leads III, aVF, T-wave changes
Lung	57	M	6664.14	1680.00	52.22	Sinus tachycardia, ST-depression in leads I, II, V2-V5,
Colon	74	F	7130.86	2314.00	21.27	Sinus rhythm, T-wave changes
Esophageal	75	M	7138.29	649.70	41.63	Sinus rhythm, T wave inversion in leads V3-V5, Frequent atrial premature beats, Low-voltage QRS
Lung	48	M	7199.41	768.60	26.97	Sinus tachycardia, T wave inversion in leads I, II and V3-V6, rSr'-pattern in V1, Prolonged QT interval
Lung *	68	M	7220.40	**115.70**	**7.43**	Sinus rhythm, ST change
Gastric	76	M	7376.89	5734.00	25.12	Sinus rhythm, T wave inversion in leads V3-V5
Lung	75	M	7383.61	591.10	36.18	Sinus rhythm, Incomplete right bundle branch block, T-wave changes
Liver	66	M	7424.69	20582.00	21.86	Sinus tachycardia, Shortened PR-interval, T-wave changes
Esophageal	67	M	7493.66	277.70	23.03	Sinus rhythm, ST-elevation in leads II, III, avF, V5-V6 and PR-segment depression, ST-depression in aVR and PR-segment elevation
Gastric	71	M	7594.90	5445.00	27.12	Sinus rhythm, rSr'-pattern in V1, Prolonged P wave duration
Liver	60	M	7638.51	677.50	**6.63**	ST-depression in leads III, avF and T wave inversion

[#] The normal value ranges of pro-BNP were **0-125** pg/ml, < 125 pg/ml, excluding chronic heart failure, and < 300 pg/ml, excluding acute heart failure. The optimal diagnostic value for acute heart failure was < 50 years old, > 450 pg/ml, or aged 50–75, > 900 pg/ml, or over 75 years old, > 1800 pg/ml and glomerular filtration rate (GFR) < 60 ml/min, > 1200 pg/ml

[&] The normal value ranges of c-TnT were **0–14** pg/ml, 15–52 pg/ml for myocardial injury, and > 52 pg/ml indicated acute myocardial injury

+ Other tests indicated calcified plaques in aortic and coronary artery walls

* Hypertension Grade 2 (high risk)

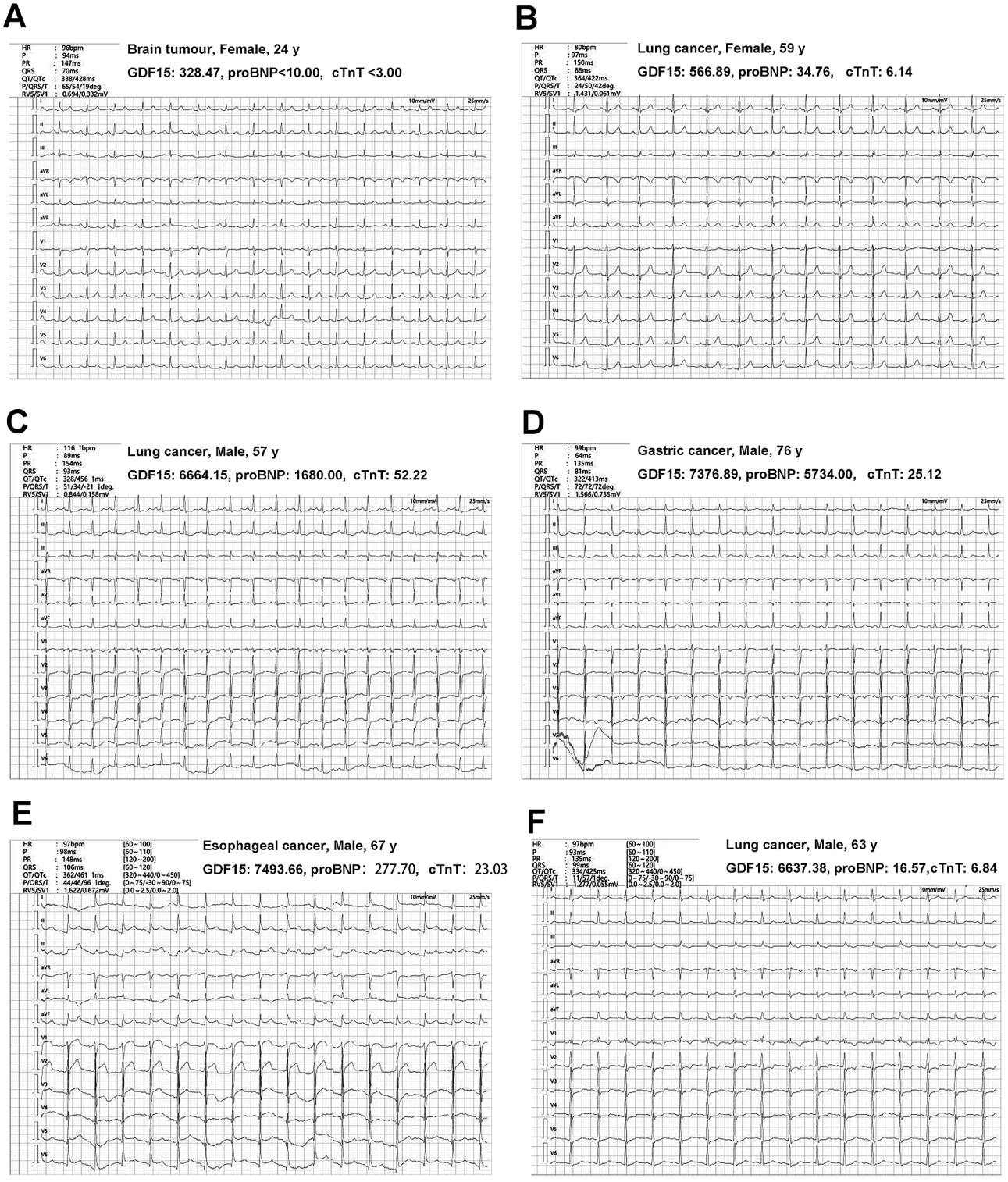

Fig. 7 ECG Outcomes Related to Serum GDF15 Concentrations in Cancer Patients. This figure shows ECG findings in cancer patients, categorized by serum GDF15 levels. Panels **(A)** and **(B)** demonstrate patients with low GDF15 levels and corresponding low c-TnT and NT-proBNP levels, resulting in normal ECG outcomes. In contrast, panels (**C**-**F**) present cases with elevated GDF15 levels alongside more complex c-TnT and NT-proBNP readings, showing a heightened incidence of ECG abnormalities. All ECG recordings were conducted at a standard speed of 25 mm/s

leads III and avF, along with T wave inversion among other abnormal alterations (Fig. 7F; Table 2). Another significant observation was made in a patient with an exceptionally high serum GDF15 level (7493.66 pg/ml); while the c-TnT (23.03 pg/ml) and NT-proBNP (277.70 pg/ml) levels were not markedly elevated, the ECG indicated serious myocardial ischemia, as evidenced by ST segment elevation in leads II, III, avF, V5-V6 (Fig. 7E).

Discussion

Cardiovascular disease and cancer are the two leading causes of death worldwide [23]. Accumulating clinical evidence demonstrates the increased risk of developing cardiac disease during cancer treatment. Cancer incidence and mortality were significantly increased in patients with heart failure [24, 25]. Addressing the cardiotoxic effects associated with anti-cancer therapies represents a formidable challenge currently confronting cardiologists and oncologists [26]. This underscores the need for a reliable serum biomarker for monitoring cardiovascular toxicity during cancer treatment. Research indicates that elevated GDF15 levels are linked to a spectrum of cardiovascular conditions, including myocardial hypertrophy, heart failure, atherosclerosis, and endothelial dysfunction. Moreover, GDF15 has been shown to precipitate cachexia and provide protection against obesity and insulin resistance in murine models [9, 27]. Notably, cardiovascular diseases, heart failure, and organ failure emerge as the prevalent clinical manifestations of cachexia induced by malignant tumors [28].

In this study, we elucidated the role of DEGs across various DSTs utilizing the TCGA database, highlighting 48 secreted proteins as potential serum biomarkers. Subsequent analyses, leveraging the GEO database, allowed us to delve into DEGs pertinent to cardiomyopathy. This dual-disease model approach culminated in the identification of five extracellular molecules, with GDF15 emerging as a notably significant biomarker for clinical detection.

Despite the variability observed in GDF15 immunohistochemical staining across normal and cancerous tissues, serum levels of GDF15 in patients with tumors significantly exceeded those in healthy controls. This disparity might be attributed to the limited sample size of healthy individuals ($n=30$) and the complexity surrounding the treatment histories of cancer patients, potentially influencing GDF15 expression. Nonetheless, GDF15 demonstrated robust diagnostic utility across a spectrum of cancers, particularly standing out in DSTs where its levels were markedly elevated, aligning with findings from prior research [29].

Our results suggested an aberrant expression of GDF15 in both cancerous conditions and cardiomyopathy, posing the question of its utility as a marker for cardiac dysfunction induced by cancer treatments. While existing studies suggest GDF15's potential as a predictive marker for CTRCD [18, 30], our analysis extends this narrative by showing a correlation between increased GDF15 levels and enhanced cardiac markers across a pan-cancer cohort. Notably, patients exhibiting the highest GDF15 serum levels also displayed elevated cardiac dysfunction markers but succumbed shortly after testing, underscoring the marker's prognostic significance. Specifically, the patient with the highest serum GDF15 concentration (7937.295 pg/ml) presented with significantly high levels of c-TnT (342.40 pg/ml) and NT-proBNP (4348.00 pg/ml), yet died merely two days following testing. Similarly, another patient, registering extremely high GDF15 levels (7804.815 pg/ml) alongside a history of premature cardiac beats and serum levels of c-TnT (15.24 pg/ml) and NT-proBNP (1265.00 pg/ml), succumbed within a month following discharge. Through medical records review, it was found that these two patients with extremely high level of GDF15 were terminal-stage (stage IV) patients with multiple organ failure caused by bone metastasis and cachexia, which may be an important cause of their death. In contrast, when analyzing lung and liver cancer patients independently, the link between GDF15 levels and cardiac biomarkers appeared to be less pronounced. This reduced correlation suggests that the majority of these cancer patients may not exhibit pronounced cardiomyopathy post-treatment, with most cardiac indicators values falling into the normal range, except for few samples showing aberrant expressions, thereby influencing the overall statistical outcomes.

ECG has historically been critical for the diagnosis and management of cardiac injury, cardiomyopathy, and cardiovascular toxicity [22, 31, 32]. In this study, we observed the ECG patterns in patients exhibiting varying levels of GDF15 expression. Generally, we noted a correlation where elevated GDF15 levels were associated with increased c-TnT and NT-proBNP levels, alongside more pronounced arrhythmic alterations (such as sinus bradycardia) and ischemic changes (including ST segment and T-wave variations). However, there were instances where GDF15 levels were markedly high, while c-TnT and NT-proBNP levels remained within normal limits or did not show significant elevation, yet the ECG demonstrated substantial changes.

It is important to acknowledge certain limitations: not every patient had their ECG recorded in close proximity to the blood collection time, leading to disparate data without a temporally-proximal correlation. Echocardiography is common monitoring methods of CTRCD according to *the Guidelines of ESC* [33]. Artificial intelligence electrocardiogram served as a screening tool to detect a newly abnormal LVEF (left ventricular ejection fraction) after anthracycline-based cancer therapy [34]

and lack of LVEF evaluation is indeed a limitation of this study. GDF15 has been proved to be a keen pro-inflammatory factor in many studies, and both cardiac disease and cancer have links to inflammation. The results of GDF15 and cardiac biomarkers may be the case, but whether the results can be applied accurately and specifically is certainly worth discussing. In addition, cancer subtypes and comorbidities may affect the expression of GDF15.

In conclusion, this hypothesis generating study attempts to reinforce the association between GDF15 expression and both cancer and cardiac damage after chemotherapy, underscoring its diagnostic efficacy in cancer patients and its potential in monitoring cardiovascular toxicity. While GDF15 levels generally correlate with traditional cardiac markers, the study reveals instances of discordance, suggesting a complementary role for GDF15 in the complex landscape of cancer treatment-related cardiac care.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40959-024-00263-9.

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Author contributions
Qinghai Lin designed experiments; Xiaohe Hao, Jing Kong, Xun Peng, Zhenyu Zhang and Cuiping Mao collected the laboratory results; Kun Ru, Chuanxi Zhao, Xinkai Mo and Rufei Ma provided the information of cancer patients; Qinghai Lin and Xiaohe Haoprepared Figs. 1, 2, 3, 4 and 5, Jing Kong and Rufei Ma prepared Figs. 6 and 7; Table 1, and 2. Qinghai Lin, Xiangguo Yu, Meijuan Cai and Lisheng Liu wrote the manuscript and analyzed the data; Xiaohe Hao polished the manuscript. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human samples were reviewed and approved by the Ethics Committee of Shandong Cancer Hospital and Institute, in accordance with the Declaration of Helsinki. The participants provided their written informed consent to participate in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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