Pan-cancer characterization of GDF15 as a potential prognosis and immunological biomarker

Xiaolei Li (✉ lixiaolei@ahmu.edu.cn)

First Affiliated Hospital of Anhui Medical University <https://orcid.org/0000-0002-2697-6496>

Qian Huai

The First Affiliated Hospital of Anhui Medical University

Cheng Zhu

The First Affiliated Hospital of Anhui Medical University

Yishan Du

First Affiliated Hospital of Anhui Medical University

Fang Ma

Anhui Medical University

Xu Zhang

The First Affiliated Hospital of Anhui Medical University

Hanren Dai

First Affiliated Hospital of Anhui Medical University

Hua Wang

First Affiliated Hospital of Anhui Medical University

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Abstract

Background Growth/differentiation factor 15 (GDF15) is a member of the TGF- β superfamily whose expression is increased in response to cellular stress and disease. Although emerging cell-or animal-based evidence supports the relationship between GDF15 and cancers, systematic pan-cancer analysis of GDF15 remains unavailable. Thus, we aimed to explore the prognostic and immunological roles of GDF15 across different types of tumors. **Methods** A comprehensive analysis of GDF15 in 33 types of cancer was performed based on the TCGA database. This involved an analysis of mRNA expression profiles, genetic alterations, methylation, prognostic values, immune infiltration analysis, potential biological pathways. Moreover, both the gain and loss of function strategies were used to assess the function of GDF15 in cell lines of hepatocellular carcinoma (HCC). **Results** GDF15 is highly expressed in most types of cancers, and there is a significant correlation between the expression of GDF15 and the prognosis of cancer patients. We have observed that GDF15 promotes the proliferation and invasion of HCC cell lines. Subsequently, methylation analyses suggested that high GDF15 expression likely resulted from hypomethylation, and immune infiltration analysis showed that GDF15 may have an impact on the changes in the tumor microenvironment. Moreover, enrichment analysis revealed that TGF- β signaling and metabolism pathways were involved in the functional mechanisms of GDF15. **Conclusions** Our unpublished pan-cancer analysis of GDF15 offers a relatively comprehensive overview of the oncogenic roles of GDF15 in multiple human cancers. GDF15 may prompt HCC cellular proliferation, invasion and metastasis. All of these provides solid basement and will promote more advanced understanding the role of GDF15 in tumorigenesis and development from the perspective of clinical tumor samples and cells.

Background

Cancer can manifest in hundreds of different forms depending on the location of the tumor, the origin of the cells, and the spectrum of genomic changes that promote tumorigenesis and affect the response to therapy (Cancer Genome Atlas Research et al., 2013). Identification and characterization of novel pan-cancer genes are crucial to better understand the extremely complex process of tumorigenesis. A lot of databases especially those based on The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets from multiple cancers are available for more detailed downstream pan-cancer analysis (Clough and Barrett, 2016; Wang et al., 2016). Thus, efforts to explore novel biomarkers for the diagnosis of cancers and the prediction of cancer therapeutic effect are critical for tumor control.

Growth/differentiation factor15 (GDF15) (a member of the TGF- β superfamily, also known as MIC-1 and NAG-1), is expressed in multiple tissues throughout the body and circulates in the bloodstream of healthy humans (Bootcov et al., 1997). GDF15 functions molecularly as a cytokine and growth factor. Under normal physiological conditions, circulating levels of GDF15 are in the range of 0.1–1.2 ng/ml; however, levels of GDF15 are markedly elevated in humans during cellular stress, such as in cardiac and renal failure (Ho et al., 2013; Stiermaier et al., 2014), chronic liver disease (Koo et al., 2018), various cancer (Welsh et al., 2003), chronic inflammatory diseases (Brown et al., 2007) and mitochondrial diseases (Tsai et al., 2018; Wang et al., 2021). Moreover, upregulation of GDF15 causes a dramatic

reduction in food intake and weight loss (Wischhusen et al., 2020; Wang et al., 2021). These observations have led to the supposition that GDF15 does not have a normal, physiological role but is, instead, secreted as an adaptive response to disease. The role GDF15 to develop and progress cancer is complicated and remains unclear. GDF15 plays a prominent role in cancer, exhibiting both pro- and antitumorigenic effects, although the latter are reported much less often than the former (Lodi et al., 2021). In the tumor milieu, GDF-15 acts on stromal cells and immune cells and promotes tumor growth by activating several mechanisms (Lodi et al., 2021); However, much less is known about the action of GDF-15 on immune cells in the TME. Understanding the actions of GDF-15 on immune cells and the mechanism involved in the immune escape of tumor cells in the presence of GDF-15 would assist in the development of improved therapeutics.

Most studies about the role of GDF15 in tumors have been limited to a specific type of cancer; however, there still remains lack of pan-cancer evidence that demonstrates the correlation between GDF15 and divergent cancer types based on big clinical data. We thus utilized multiple databases, including TCGA, cBioPortal, and Human Protein Atlas (HPA), to analyze GDF15 expression levels and their relationship with prognosis in different types of tumor. We also included a group of factors, such as genetic alteration, survival status, DNA methylation, genetic alteration, immune infiltration, and relevant cellular pathway, to investigate the potential molecular mechanism of GDF15 in the pathogenesis or clinical prognosis of different cancers. Our unrepresented pan-cancer analysis provides a deep understanding of the functions of GDF15 in oncogenesis in different cancers and identifies strategies that may be used to promote collaborative activities in the context of immunotherapy.

Materials And Methods

Gene expression analysis

To obtain the expression difference of *GDF15* between tumor and adjacent normal tissues for the different tumors or specific tumor subtypes in the TCGA project, we input GDF15 in the 'Gene_DE' module of TIMER2 (tumor immune estimation resource, version2) web (<http://timer.cistrome.org/>). For those without normal tissue matched as controls in TIMER2 database, we applied the 'Expression analysis Box Plots' module of the GEPIA2 (Gene Expression Profiling Interactive Analysis, version 2) web server (<http://gepia2.cancer-pku.cn/#analysis>) to obtain box plots of the expression level difference between the tumor tissues and corresponding normal tissues from GTEx (Genotype-Tissue Expression) database (Tang et al., 2019). In addition, we selected the 'Pathological Stage Plot' module of GEPIA2 to obtain violin plots of the GDF15 expression level in different pathological stages. Moreover, we used the UALCAN portal (<http://ualcan.path.uab.edu/analysis-prot.html>) to perform protein expression analysis of the CPTAC (Clinical proteomic tumor analysis consortium) dataset (Chen et al., 2019).

Survival prognosis analysis

To obtain the OS (Overall survival) and DFS (Disease-free survival) significance map data of GDF15 in all TCGA tumors, the 'Survival Map' module of GEPIA2 was used (Tang et al., 2019). Judging from cutoff-

high (50%) and cutoff-low (50%) values, we divided all cases into two groups, namely, high-expression group and low-group. For hypothesis test, the log-rank test was used, and use the 'Survival Analysis' module of GEPIA2 to obtain the survival plots.

Genetic alteration analysis

Firstly, we logged into the cBioPortal web (<https://www.cbioportal.org/>) (Cerami et al., 2012; Yaffe, 2013), and clicked the 'TCGA Pan Cancer Atlas Studies' in the 'Quick select' section. Secondly, entered 'GDF15' for queries of the genetic alteration characteristics of GDF15. We select 'Cancer Types Summary' module to observe the alteration frequency, mutation type and CNA (Copy number alteration) in all TCGA tumors. By use the 'Mutations' module, the mutated site in the protein structure or the 3D (Three-dimensional) structure can be showed. We also used the "Comparison" module to analysis the survival progression differences for the TCGA cancer cases that with or without GDF15 genetic alteration. Kaplan–Meier plots with log-rank *P*-value were appeared as well.

MTT assay

Dispense 5×10^3 cells into per well of the 96-well plates in a total volume of 100 μ l. Incubate at 37 °C for 48–72 h in a humidified, 5% CO₂ atmosphere. Add 20 μ l per well of CellTiter 96®AQueous One Solution Reagent. And then, continue incubate at 37°C for 1–4 h in the same condition. Using a 96-well plate reader to record the absorbance at 490nm. Each experiment was performed with three replicates (Li et al., 2017).

3D Matrigel Cell Culture

Cancer cells were grown to 80% confluence in a monolayer on plastic tissue culture dishes before trypsinization and collection by centrifugation (Li et al., 2013). Matrigel (BD Biosciences) was thawed on ice overnight. 3D cultures were prepared by mixing 5×10^4 cancer cells with 1 ml of Matrigel and plating the mixture in 6-well dishes. Following a 1 h incubation at 37°C, the Matrigel had polymerized. Medium (1–2 ml) containing 10% FBS was added to the top of the solidified Matrigel-cell mix and was replaced every other day. Cultures were routinely grown for two weeks in Matrigel. Each experiment was performed in triplicate and each experiment was repeated twice. Each bar represents the mean (plus standard error) of six independent experiments.

Wound healing assay

Cell migration was assessed by measuring the movement of cells into a scraped, acellular area created using a 200 μ l pipette tube, and the extent of wound closure was observed after 18 and 36 h and photographed under a microscope (Li et al., 2013).

Transwell assay

For the transmigration assay, cells (5×10^3 to 1×10^4) were plated in the top chamber of uncoated membranes (6-well inserts, 8 μ m; Corning). For the invasion assay, 1×10^4 to 1.5×10^4 cells were seeded in the top chamber on Matrigel-coated (BD Biosciences) membranes (Li et al., 2013). For both assays,

cells were seeded in medium without FBS in the top chamber, and medium containing 10% FBS was placed in the lower chamber to stimulate migration or invasion. Cells were incubated for 24 to 36 hours before they were stained with Giemsa and counted.

Immune infiltration analysis

To explore the correlation between *GDF15* expression and immune infiltrates we applied the 'Immune-Genes' module of the TIMER2 (tumor immune estimation resource, version 2, <https://timer.cistrome.org/>) tool. And then, we selected the CD8 + T-cells and cancer-associated fibroblasts to analysis. To estimate the immune infiltration the TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, MCPOUNTER and TIDE algorithms were selected. Through purity-adjusted Spearman's rank correlation test, the *P*-values and partial correlation (cor) values were obtained. The data were shown as a heatmap and scatter plot.

GDF15-related gene enrichment analysis

We used STRING website (<https://string-db.org/>) for the subsequent analysis of protein-protein interaction network. The main parameters were: minimum required interaction score ["Low confidence (0.150)"], meaning of network edges ("evidence"), max number of interactors to show ("no more than 50 interactors" in 1st shell) and active interaction sources ("experiments"). GEPIA2 was used to obtain the top 100 *GDF15*-correlated genes based on the datasets of all TCGA tumor and normal tissues. Then, we conducted a pairwise gene-gene Pearson correlation analysis between *GDF15* and the selected genes. The *P*-values and the correlation coefficient (R) were calculated and are indicated in the corresponding figure panels. Heatmap representation of the expression profile for the selected genes contains the partial correlation (cor) and *P*-value in the purity-adjusted Spearman's rank correlation test. GO enrichment (including BP, CC, and MF) and KEGG pathway analyses of these genes were performed using Metascape and visualized by the R package ggplot2. An adjusted $p < 0.05$ was determined to be statistically significant.

Statistical analysis

Alterations in *GDF15* expression levels in cancer tissues and normal tissues were estimated using *t*-tests. For survival analysis, the HR and *p* value were calculated using univariate Cox regression analysis. Kaplan-Merier analysis was used to investigate the survival time of patients stratified according to high or low levels of the *GDF15* expression. $p < 0.05$ was set as the significance threshold for all statistical analyses.

Results

The data of gene expression analysis of *GDF15*

We sought to define the oncogenic role of human *GDF15* (NM_004864.4 for mRNA or NP004855.2 for protein, **Figure S1a**). In the first place, we analyzed the expression level of *GDF15* in various cells and tumor tissues. As is presented in **Figure S1b, c**, *GDF15* has broadly expressed in tumor tissues obtained

from patients with different tumor types, and also in tumor cell lines of various tissues, based on the Human Protein Atlas (HPA).

To determine the expression level of *GDF15*, we applied the TIMER2 database. As shown in **Fig. 1a**, the expression of *GDF15* in the tumor tissues of BRCA, CHOL, COAS, ESCA, HNSC, LIHC, LUAD, PPAD, READ, STAD, THCA and UCEC is higher than that of corresponding controlled normal tissues. However, the expression of *GDF15* in the tumor tissues of KICH, KIRC, KIRP, and LUSC is lower than that of corresponding controlled normal tissues.

For those without normal tissue matched as controls in TIMER2 database, we utilized the GTEx dataset to further evaluate the disparity of *GDF15* expression between normal and tumor tissues. *GDF15* definitely expressed at a higher level in tumor tissues than in the corresponding normal tissues in ACC, TGCT, UCS, OV (**Fig. 1b**), and SKCM (**Figure S2a**), however, the expression of *GDF15* in the tumor tissues of LAML is lower than that of corresponding controlled normal tissues (**Figure S2a**). Regardless, we did not observe that evident difference in DLBC, LGG, SARC, and THYM (**Figure S2b**). The total protein of *GDF15* obtained from the CPTAC datasets revealed elevated expression trend of *GDF15* in the tissues derived from ovarian cancer, colon cancer, UCEC, and LUAD (**Fig. 1c**) than that in normal tissues, but not significant. To further evaluate *GDF15* expression at the protein level, we analyzed IHC results provided by the HPA database and compared the results with *GDF15* gene expression data from TCGA. As shown in **Figure S3a-h**, the results of analysis of data from these two databases were consistent with one another. Normal tissue samples had weak *GDF15* staining, while tumor tissues had moderate or strong staining.

To unveil the correlation between *GDF15* expression level and the pathological stages of tumors, the “Pathological Stage Plot” module of GEPIA2 was used. The result pointed to a strongly tight correspondence in BRCA, DLBC, HNSC, KIRP, THCA and TGCT (**Fig. 1d, Figure S4a**), but not in other cancer types (**Figure S4-e**).

The results of survival analysis

Judging from the expression levels of *GDF15*, we divided all cases into two groups, namely, high-expression group and low-expression group. Then, taking advantage of TCGA and GEO datasets, we further investigate the relationship between *GDF15* expression and the prognosis of different tumors. As elucidated in **Fig. 2a**, in TCGA project, we found that the higher *GDF15* expressed, the poorer prognosis of overall survival (OS) will be in GBM, LGG and MESO. Disease-free survival (DFS) analysis data (**Fig. 2b**) revealed a similar tendency that higher *GDF15* expression was connected with poorer prognosis in GBM and LGG. In contrast, lower expression of the *GDF15* was correlated with poorer OS in COAD (**Fig. 2a**).

Next, with the aid of Kaplan-Meier plotter tool, we performed survival data analysis, finding that higher expression *GDF15* was connected with poorer OS prognosis in LIHC, HNSC and KIRP. Conversely, higher *GDF15* expression level was associated with better OS for STAD, CESC, BLCA, UCEC and LUAD (**Figure S5a**). As well, we performed survival data analysis, finding that higher expression *GDF15* was connected

with poorer relapse-free survival (RFS) prognosis in LIHC, PAAD and TGCT. Nevertheless, in READ, SARC and STAD cancer patients, lower expression GDF15 patients had poorer RFS prognosis (**Figure S5b**).

Correlation between methylation and expression of GDF15

Firstly, we calculated the levels of correlation between GDF15 promoter methylation using the cBioPortal web and identified significant correlations between gene expression and methylation in 22 tumors. In almost all types of tumors, there were negative correlations between GDF15 expression and promoter methylation levels except in patients with TGCT (**Figure S6**).

To further elucidate the abnormal upregulated mechanisms of *GDF15* in HCC tissues, we also explored the correlation of GDF15 expression levels and their methylation status by various methods. First, differential expression analysis of three DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B) between GDF15^{high} and GDF15^{low} groups of HCC showed that expression of the DNMT3A, rather than DNMT1 and DNMT3B, in GDF15^{high} group was markedly increased compared with that in GDF15^{low} group (**Fig. 3a**). Second, the analysis of UALCAN showed that GDF15 had a trend of higher methylation level in normal tissues than in tumor tissues of LIHC, HNSC and BLCA (**Fig. 3b**). Similarly, DiseaseMeth version 2.0 analysis demonstrated that the methylation of GDF15 was significantly higher in normal tissues than in tumor tissues of LIHC, HNSC and BLCA (**Fig. 3c**). Additionally, we found several methylation sites in the DNA sequences of GDF15 that were negatively associated with their expression levels (Fig. 3d). Third, the differential methylation regions related to GDF15 were presented as heatmaps (**Figure S7**). These data suggested that DNA methylation might involve in regulating GDF15 gene expression, which deserve more in-depth research.

The data of genetic alteration in GDF15

We analyze the genetic alteration status of GDF15 in TCGA cohorts, which included different subtypes of tumor samples. As presented in **Fig. 4a**, GDF15 alteration types include mutations, amplifications, fusions, deep deletion and multiple alterations. The highest alteration frequency of GDF15 (>6%) appears for patients with ovarian cancer with “amplification” as the primary type of CNA. The “mutation” type of CNA was the primary type in the skin cutaneous melanoma cases, which show an alteration frequency of ~3% (**Fig. 4a**). As presented in **Fig. 4b**, we inclusively exhibited the types, sites and case numbers of GDF15 genetic alteration. Detailed analysis reveals that the main type of genetic alteration for GDF15 was missense mutation. For example, as a missense mutation, R217H alteration in the TGF-beta domain was found in 1 case of COAD (**Fig. 4b**). The 3D structure of GDF15 protein with R217H site included were shown in **Fig. 4c**. Additionally, we investigated the correlation between genetic alteration of GDF15 and the clinical feature of survival prognosis in different type of cancers. As is shown in Fig. 4d, compared with tumor cases with GDF15 alteration, those cases accompanied without altered GDF15 have better prognosis in progression-free ($P=0.017$) survival, but not in overall ($P=0.326$), disease-specific ($P=0.0888$), and disease-free ($P=0.0592$) survival.

Tumor mutational burden (TMB) is a promising pan-cancer predictive biomarker, which could guide immunotherapy in the era of precision medicine (Steuer and Ramalingam, 2018). Next, we tried to figure out the correlation between *GDF15* expression level and the TMB. We found that *GDF15* expression was positively associated with TMB in THYM ($P=0$), UCEC ($P=6.65\text{e-}14$), STAD ($P=0.0014$), SARC ($P=0.008$), LGG ($P=1.83\text{e-}13$), KIRC ($P=0.0102$), and ESCA ($P=0.003$) but negatively associated with BRCA ($P=5.82\text{e-}07$) and LUAD ($P=9.5\text{e-}10$), as illustrated in **Figure S8**. In addition, microsatellite instability (MSI), also an indicator of genetic instability, can be helpful to screen patients for immunotherapy. We further discovered that the *GDF15* level was positively associated with MSI in UCEC ($P=0.0055$), STAD ($P=0.015$), KIRC ($P=0.025$), and ESCA ($P=0.0103$) but negatively correlated with COAD ($P=0.048$) and READ ($P<0.001$), as shown in **Figure S9**.

The function of GDF15

To better understand the function of *GDF15*, we selected liver cancer cell lines for study. We firstly performed the qPCR and western blotting to study the expression of *GDF15* in HCC cells. Compared with Lo2 cells, which are recognized as immortalized human normal liver cells, HCC cells had higher expression of *GDF15* mRNA together with its protein (**Figs. 5a, b**).

Next, we manipulated *GDF15* expression in cells by means of recombinant protein and neutralizing antibody of *GDF15*. As shown in **Figs. 5c, e**, Huh7 cells treated with recombinant *GDF15* (r*GDF15*) led to a significant increase in anchorage-independent growth and promoted the overall growth rate of the cells. Conversely, HepG2 cells treated with *GDF15* antibody (a-*GDF15*) to get rid of *GDF15* proteins significantly reduced anchorage-independent growth and led to lower growth rates compared with cells treated with control antibody (**Figs. 5c, e**).

Moreover, wound healing and transwell assays were also executed in order to identify the migration and invasion capacity. Results showed that, compared with control, Huh7 cells treated with r*GDF15* had a more invasion cells and faster wound closure. However, HepG2 cells treated with a-*GDF15* had remarkable reduction of migratory and invasive capacity compare to control treatment (**Figs. 5d, f**). Combining related experiment and results, we can propose that *GDF15* promotes HCC cell proliferation, migration, and invasion capacity in vitro.

The data of immune infiltration analysis

Infiltrating immune cells in tumor are recognized as the predominant elements of microenvironment within tumor, which make differences to tumorigenesis and development or metastasis. Thus, we performed a pan-cancer analysis of the correlation between the *GDF15* expression and the immune infiltration level based on the TIMER database. As shown in **Figure S10a**, *GDF15* expression was significantly correlated with the abundance of infiltrating immune cells: B cells in 17 types of cancer, CD4+ T cells in 16 types of cancer, CD8+ T cells in 13 types of cancer, macrophages in 15 types of cancer, neutrophils in 16 types of cancer, and DCs in 20 types of cancer. Next, we used the xCell online tool to examine the relationship between *GDF15* expression and the infiltration of different types of immune cell

subtypes. Among 38 subtypes of immune cells, we found that the *GDF15* expression negatively and significantly correlated with these subtypes in BLCA, CESC, CHOL, COAD, TGCT and PAAD (**Figure S10b**). Macrophages and endothelial cells were most positively correlated with the *GDF15* expression on these cancers (**Figure S10b**). The estimate package was used to analyze the stromal score, microenvironment score, and immune score of each tumor sample and determine the relationship between *GDF15* expression, stromal score, microenvironment score, and immune score in 33 types of cancer. Results showed that the stromal scores of these cancers were most positively correlated with the *GDF15* expression. By contrast, the immune scores of these cancers were most negatively correlated with the *GDF15* expression, as illustrated in **Figure S10b**.

As is known, cancer-associated fibroblasts play a regulatory role towards various tumor-infiltrating immune cells function (Chen and Song, 2019; Sahai et al., 2020). In this part, we set to define the underlying relationship between infiltration status of diverse immune cells and *GDF15* expression across diverse cancer types from TCGA, the TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, the XCELL, MCPOUNTER, and EIPC algorithms were used. Through a series of analysis, we unveiled the statistical pattern that *GDF15* expression was positively correlated with the predictive infiltration degree of CAFs for TCGA tumors including BRCA-basal, GBM, LGG, TGCT, and THYM. On the other hand, a negative correlation was observed in ESCA and PAAD (**Fig. 6**). Next, a negative correlation between *GDF15* expression and infiltration of CD8⁺ T cells was observed in most tumor types of cancer from TCGA (**Figure S11**).

Relationship between *GDF15* and immune checkpoints

Immune checkpoints maintain the activation of the immune system within the normal range. Their abnormal expression is closely related to the occurrence and development of some tumors, representing a potential treatment option for patients with various types of malignancies (Li et al., 2018; Li et al., 2019). Based on the potential correlation between *GDF15* expression and immune infiltration levels, next, we further explored the correlation between *GDF15* expression and immune checkpoints. Therefore, we conducted a correlation analysis between *GDF15* expression and immune checkpoint molecules in 33 cancer types. In particular, the expression of *GDF15* was positively correlated with the expression of a plethora of immune checkpoint molecules, such as CD274, CTLA-4, TIM3, LAG3, PDCD1, and TIGIT, in LGG, PCPG, and UVM (**Figure S12**). In contrast, in BLCA, BRCA, CESC, COAD, LUAD, and TGCT, *GDF15* expression was negatively correlated with a series of immune checkpoint molecules, such as CD274, CTLA-4, LAG3, PDCD1, and TIGIT (**Figure S12**). In summary, *GDF15* is very important in regulating tumor immunity, which might explain its influence on the prognosis and survival of patients with cancer.

Intriguingly, the genomic investigation indicated that *GDF15* was actually involved in the alteration of immune checkpoints in cancer. The general landscape of *GDF15* and immune checkpoint alteration in 33 cancer types was compactly visualized, including fusion, amplification, deep deletion, truncating, and missense mutations (**Figure S13a**). The detailed relationship between *GDF15* and each representative immune checkpoint was individually presented as indicated in **Figure S13b**. Of note, the *GDF15* alteration

showed a statistically significant co-occurrence rather than mutual exclusivity with extensive immune checkpoints, such as PDCD1 and LAG3. These findings strongly reveal that GDF15 is a potential coregulator of immune checkpoints in cancer.

The data of enrichment analysis of GDF15

To further demonstrate the molecular mechanism of *GDF15* gene in tumorigenesis, we attempted to screen out the targeting GDF15-binding proteins and the *GDF15* expression-correlated genes for a series of pathway enrichment analyses. Based on the STRING tool, we obtained a total of 50 GDF15-binding proteins, which were supported by experimental evidence. **Fig. 7a** shows the interaction network of these proteins. We also used the GEPIA2 tool to combine all tumor expression data of TCGA and obtained the top 100 genes that correlated with *GDF15* expression. As shown in **Fig. 7b**, the *GDF15* expression level was positively correlated with that of *CLDN4* (Claudin4) ($R=0.4$), *CRB3* (Crumbs cell polarity complex component 3) ($R=0.43$), *BBC3* (BCL2 binding component 3) ($R=0.35$), *ELF3* (E74 like ETS transcription factor 3) ($R=0.3$), *S100A6* (S100 calcium binding protein A6) ($R=0.24$), and *SPINK1* (Serine peptidase inhibitor Kazal type 1) ($R=0.35$) genes (all $P<0.001$). The corresponding heatmap data also showed a positive correlation between *GDF15* and the above five genes in the majority of detailed tumor types (**Fig. 7c**). An intersection analysis of the above two groups showed one common member, namely, *SPINK1* (**Fig. 7d**).

We combined the two datasets to perform KEGG and GO enrichment analyses. The KEGG data of **Fig. 7e** suggest that “TGF-beta signaling pathway” and “metabolic pathways” might be involved in the effect of GDF15 on tumor pathogenesis. The GO enrichment analysis data further indicated that most of these genes are linked to the signaling pathways or kinase complex, such as activin receptor signaling pathway, serine/threonine protein kinase complex, and others (**Figs. 7f-h**).

Discussion

It has been reported that the multifunctional GDF15 regulates in a series of cellular biological processes, such as food intake, energy expenditure and body weight in response to metabolic- and toxicity-induced stress (Wang et al., 2021). In this study, “HomoloGene” and phylogenetic tree analysis data confirmed the conservation of the GDF15 protein structure across different species, suggesting that similar mechanisms might exist for the normal physiological role of GDF15. Emerging studies have reported a functional link between GDF15 and clinical diseases, especially tumors (Ratnam et al., 2017; Lodi et al., 2021; Myojin et al., 2021). Whether GDF15 can play a role in the pathogenesis of different tumors through certain common molecular mechanisms remains to be answered. Through a literature search, we failed to retrieve any publication with a pan-cancer analysis of GDF15 from the perspective of overall tumors. We comprehensively examined the GDF15 gene in a total of 33 different tumors based on the data of TCGA database and the molecular features of gene expression, genetic alteration, or DNA methylation. However, additional studies will be needed to further explore its function in different cellular context.

Growing number of studies have focused on the analysis of the function of GDF15 in diseases, including cancer (Wischhusen et al., 2020; Myojin et al., 2021). Nevertheless, it remains poorly understood whether GDF15 is involved in the oncogenesis of certain tumor types, or rather in more common pathways driving tumor pathogenesis. Thus, here we set out to perform a pan-cancer analysis of GDF15. Our comprehensive approach included the exploration of the GDF15 gene expression in a total of 33 different tumors based on the data of TCGA. We also systematically collected and integrated immune cell infiltration data using TIMER databases, as well as other molecular features, such as DNA methylation and genetic alterations.

DNA methylation is a broadly-investigated epigenetic modification that has been considered as a heritable and reversible change. DNA methylation regulates the gene expression without altering the sequence of DNA and generally suppresses the gene expression by changing chromatin structure, DNA stability, and DNA conformation (Esteller, 2003; Koch et al., 2018). Cancer cells are characterized by aberrant DNA methylation (e.g., genome-wide hypomethylation and site-specific hypermethylation), mainly targeting CpG islands in gene expression regulatory elements. In particular, the early findings that a variety of tumor suppressor genes are targets of DNA hypermethylation in cancer led to the proposal of a model in which aberrant DNA methylation promotes cellular oncogenesis through TSGs silencing (Nishiyama and Nakanishi, 2021). The present study showed that DNA methylation of GDF15 was downregulated in most common cancers, which is consistent with the upregulation of the GDF15 expression. However, the relationship between DNA methylation and GDF15 expression warrants more in-depth study.

The role of GDF15 in the biology of cancer has been studied using a number of different approaches, which have yielded somewhat contradictory results. GDF15 plays a prominent role in cancer, exhibiting both pro- and antitumorigenic effects, although the latter are reported much less often than the former (Lodi et al., 2021). The tumor microenvironment (TME) is a tumor-promoting setting that tumor cells use to evade immune surveillance (Quail and Joyce, 2013). The presence of the TME significantly influences therapeutic response and clinical outcome. Various components participate in the formation of the TME, including cancer-associated fibroblasts, lymphocytes, endothelial cells, mesenchymal stem cells, and the extracellular matrix. GDF15, a member of the TGF β superfamily, plays multiple roles in a wide variety of cellular processes. GDF15 affects both stromal cells and immune cells in the TME. In this study, we investigated the correlation between the GDF15 gene and immune cells as well as stromal infiltration with the CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, MCPOUNTER, and EPIC algorithms. GDF15 expression had functions associated with CAFs (**Fig. S4**) and CD8 + T-cell infiltration (**Fig. S5**) in different cancers. CAFs serve pro-tumorigenic roles in the TME by secretion of various growth factors, cytokines, and chemokines and by degradation of the extracellular matrix. CD8 + T cells play an important role in immune-related tolerance and immunosuppression within the TME. These associations between GDF15 and TME might be another reason for the prognostic implications of GDF15 in various cancers. In addition, GDF15 as an NF- κ B-regulated gene whose production by tumor cells and signaling in macrophages serves as an important promoter of early cancer development (Ratnam et al., 2017). Taken together, these results suggest that aberrant GDF15 expression might play a leading role in the TME.

The present study unveiled a complicated role of GDF15 aberrant expression in cancer progression and patient outcome that warrant further investigation. Since our study was based on bioinformatics and rely on public databases, there are major limitations. First, quality of data collection and the method used to generate the data could be inconsistent depend on the source. This could have impact on the conclusion of the some of the analysis. Secondly, the results and conclusions are not experimentally or prospectively validated in the laboratory or in clinic. Future study to validate the expression and function of GDF15 is needed.

Declarations

Acknowledgments

The results shown here are in part based upon data generated by the TCGA Research Network: <https://www.cancer.gov/tcga>.

Author contributions

X.L. conducted the analyses and wrote the manuscript. Q.H., C.Z., F. M., and Y.D. collected data, methodology and software, X.Z. helped to interpret the data, H.D. critically analyzed the study and corrected the manuscript, H.W. designed the study, interpreted the results, and revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

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Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files. All data used in this work can be acquired from the GEO and TCGA database.

Compliance with ethical standards

Conflict of interest

The authors declare no potential conflict of interest related to this study.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent was obtained from human participants by TCGA and GEO researchers.

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Figures

Figure 1

Expression level of GDF15 gene and pathological stages in different tumors. (a) The expression status of the GDF15 gene in different cancers or specific cancer subtypes. (b) The expression statuses of GDF15 in ACC, TGCT, UCS and OV in the TCGA project were compared with the corresponding normal tissues of the GTEx database. (c) Based on the CPTAC dataset, we also analyzed the expression level of GDF15 total protein between normal tissue and primary tissue of LUAD, UCEC, colon cancer and ovarian cancer. (d)

According to the TCGA data, the expression level of the GDF15 gene were analyzed by the main pathological stages (stage I, stage II, stage III, and stage IV) of BRCA, DLBC, HNSC and KIRP. Log2 (TPM +1) was utilized for log-scale. *P<0.05, **P<0.01, ***P<0.001.

Figure 2

Correlation analysis between GDF15 expression and survival prognosis of cancers in TCGA. (a) The correlation between GDF15 expression and overall survival of different tumors in TCGA datasets. (b) The correlation between GDF15 expression and disease-free survival of different tumors in TCGA datasets.

Figure 3

Methylation analysis of GDF15. (a) Differential expression of three DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B). (b) Methylation was evaluated using UALCAN. (c) Methylation was assessed using DiseaseMeth version 2.0. (d) The methylation site of GDF15 DNA sequence association with gene expression was visualized using MEXORESS. The expression of GDF15 is illustrated by the blue line in

the center of the plot. Pearson's correlation coefficients and p values for methylation sites and query gene expression are shown on the right side.

Figure 4

Mutation characteristic of GDF15 in different tumors of TCGA. (a) and (b) The alteration frequency with mutation type and mutation site of GDF15 in the TCGA tumors are displayed by the cBioPortal tool. (c) The mutation site R217H in the 3D structure of GDF15. (d) The potential correlation between mutation status and overall, disease-specific, disease-free and progression-free survival of pan-cancer conduct by the cBioPortal tool.

Figure 5

GDF15 expression is elevated in HCC cells and promotes HCC cells proliferation and invasion. (a) GDF15 expression in human HCC cell lines analyzed by qPCR. (b) GDF15 expression in human HCC cell lines analyzed by western blotting. (c) The representative pictures (left panel) and quantification (right panel) of colony numbers of indicated cells as determined by an anchorage-independent growth assay. (d) The representative pictures (left panel) and quantification (right panel) of invaded cells were analyzed using

the transwell matrix penetration assay. (e and f) MTT and wound-healing assay were subjected to detect the proliferation and migration capacity, respectively, of HCC cells.

Figure 6

Correlation between GDF15 expression and immune infiltration of cancer-associated fibroblasts. (a) EPIC, MCPCOUNTER, XCELL and TIDE algorithms were used to explore the correlation between the expression level of the GDF15 gene and the infiltration level of cancer-associated fibroblasts. (b-j) The relationship of GDF15 and infiltration level of cancer-associated fibroblasts across BRCA, GBM, LGG, ESCA, PAAD, LIHC, TGCT, PCPG, and THYM.

Figure 7

GDF15-related gene enrichment analysis. (a) We first obtained the available experimentally determined GDF15-binding proteins using the STRING tool. (b) Using the GEPIA2 approach, we also obtained the top 100 GDF15-correlated genes in TCGA projects and analyzed the expression correlation between GDF15 and selected targeting genes, including BBC3, CLDN4, CRB3, ELF3, S100A6 and SPINK1. (c) The corresponding heatmap data in the detailed cancer types are displayed. (d) An intersection analysis of

the GDF15-binding and correlated genes was conducted. (e-h) Based on the GDF15-binding and interacted genes, both KEGG pathway and GO analysis were also performed.

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