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ORIGINAL ARTICLE

Expression and prediction of genes related to IGF2BP3 in gastric cancer*

Yulong Li[1] (✉), Yang Yang[2], Ruifang Sun[3]

[1] Department of gastroenterology, Shaanxi Provincial People's Hospital, Xi'an 710068, China
[2] School of Public Health, Shaanxi University of Chinese Medicine, Xianyang 712046, China
[3] Department of Pathology, School of Basic Medical Sciences, Xi'anJiaotong University Health Science Center, Xi'an Jiaotong University, Xi'an 710061, China

Abstract

Objective Gastric cancer (GC) is one of the most prevalent cancers worldwide and is associated with high morbidity and mortality rates. The IGF2 mRNA-binding protein (IGF2BP) participates in a variety of cancers. The aim of this study was to analyze the expression of IGF2BP3 and explore the genes related to *IGF2BP3* in GC.

Methods Bioinformatics software was used to analyze the expression of *IGF2BP1*, *IGF2BP2*, and *IGF2BP3* in tumors, and the expression of IGF2BPs in the GSE118897 dataset. Immunohistochemistry was performed to detect the protein level of IGF2BP3 in GC samples. cBioPortal was used to query gene alteration of IGF2BP3. LinkedOmics was used to identify genes related to *IGF2BP3*.

Results Sangerbox analysis showed that the expression of all IGF2BP family members was higher in GC. cBioporta analysis showed that gene alteration of IGF2BP3 in stomach adenocarcinoma included mutation and amplificatio. LinkedOmics analysis showed that many genes were correlated with IGF2BP3, such as *PLAGL2*, *GET4*, *IGF2BP1*, *HMGA2*, *CLDN6*, *HOXC13*, *SMARCA2*, *TMEM66*, *CIRBP*, *NFIX*, *SLC25A12*, and *CYB5D2*.

Conclusion In this study, we founded that IGF2BP3 was overexpressed in GC. Furthermore, this study identified potential genes related to IGF2BP3 in GC, which should be studied further.

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Key words: gastric cancer (GC); IGF2 mRNA-binding protein 3 (IGF2BP3); bioinformatics analysis

Gastric cancer (GC) is one of the most prevalent cancers worldwide and is associated with high morbidity and mortality rates. There were an estimated 1,033,700 new stomach cancer cases and 782,700 stomach cancer-related deaths in 2018 [1]. Since the symptoms of GC are not obvious, most patients are diagnosed at intermediate and advanced stages when surgery is no longer an option. The prognosis for GC is poor, with patients in the advanced stage having a mean total survival of 10–12 months [2]. Therefore, identification of novel biomarkers is vital for the early diagnosis of GC.

The conserved IGF2 mRNA-binding protein (IGF2BP) family includes the genes *IGF2BP1*, *IGF2BP2*, and *IGF2BP3*, which encode a family of RNA-binding proteins that regulate their target genes. IGF2BP proteins play important roles in development, the nervous system, and cancer, and act as essential modulator in cell growth and differentiation [3]. IGF2BP3, also known as IMP3, binds to RNA and regulates the expression of target mRNAs involved in carcinogenesis. The expression of *IGF2BP3* may serve as a predictor of bladder cancer, since the protein expression of IGF2BP3 has been associated with advanced tumor stage, grade, and recurrence [4]. *IGF2BP3* has been suggested as a poor prognostic marker in gastric tumors [5].

In this study, we used bioinformatics to analyze the expression of IGF2BPs in GC. We selected IGF2BP3 for further study and performed immunohistochemistry (IHC) to detect the protein level of IGF2BP3 in GC. Furthermore, genes related to *IGF2BP3* in GC were analyzed using LinkedOmics. Our results suggest that *IGF2BP3* represents a promising therapeutic target for GC.

✉ Correspondence to: Yulong Li. E-mail: liyulong0639@126.com
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Materials and methods

SangerBox analysis

SangerBox (http://sangerbox.com/) was used to detect the expression of IGF2BP1, IGF2BP2, and IGF2BP3 in tumors based on The Cancer Genome Atlas (TCGA) and the Genotype-Tissue expression project (GTEx). Differences were considered significant at $P < 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Gene Expression Omnibus (GEO) analysis

GEO (https://www.ncbi.nlm.nih.gov/geo/) was used to further assess the expression of IGF2BPs in GSE118897 dataset [6] in matched GC and normal tissues. Based on the results of these analyses, we selected *IGF2BP3* for further study.

UALCAN analysis

UALCAN (http://ualcan.path.uab.edu/) [7] was used to detect the promoter methylation expression of IGF2BP3.

IHC assays

Tissue microarrays were obtained from Shanghai Outdo Biotech Company (Shanghai, China). Each section was deparaffinized with xylene and hydrated using an alcohol gradient. The sections were then treated for endogenous peroxidase-blocking and antigen retrieval. The sections were incubated with rabbit anti-IGF2BP3 followed by incubation with a secondary antibody. For visualization, 3,3'-diaminobenzidine (DAB) and hematoxylin were used. Digital images were obtained using a Leica image analysis system.

Genetic alteration analysis

The cBioPortal (http://www.cbioportal.org/) [8] was used to determine the genetic alteration characteristics of IGF2BP3. We chose the "Quick select" section and entered "IGF2BP3" to check the results of the alteration characteristics of IGF2BP3 across TCGA tumors which were observed in the "Cancer Types Summary" module.

LinkedOmics analysis

The LinkedOmics database [9] (http://www.linkedomics.org) contains multi-omics and clinical data from 32 cancer types and 11,158 patients from TCGA. We used the LinkedOmics database to identify genes related to *IGF2BP3* in the TCGA stomach adenocarcinoma. Pearson's correlation test was used to analyze the results.

Results

Expression of *IGF2BPs* in GC

We detected the expression levels of *IGF2BPs* from TCGA and GTEx using SangerBox. The results showed that IGF2BP1, IGF2BP2, and IGF2BP3 were more highly expressed in stomach cancer than in normal tissues (Fig.1a–1c).

IGF2BP3 expression is higher in GC

GEO was used to further assess the expression of *IGF2BP* genes in the GSE118897 dataset. The results showed that *IGF2BP1* and *IGF2BP2* were more highly expressed in GC tissues than in the normal gastric mucosa. However, this difference was not statistically significant (Fig. 2a and 2b). Compared with the expression in the normal gastric mucosa, *IGF2BP3* in GC tissues was significantly overexpressed (Fig. 2c). Therefore, we selected *IGF2BP3* for further study. The level of *IGF2BP3* promoter methylation in stomach cancer was also analyzed using UALCAN. The data showed that the promoter methylation level of *IGF2BP3* was significantly lower in stomach cancer tissues than in the control tissues (Fig. 2d).

Expression of IGF2BP3 is higher in GC

We performed IHC assays to detect the protein expression of IGF2BP3 in GC. The results demonstrated that the expression of IGF2BP3 was higher in GC tissues than in paracarcinoma tissues (Fig. 3).

Mutation of IGF2BP3

Fig. 4 showed the gene alteration of IGF2BP3 in tumor samples of the TCGA cohorts. The type of gene alteration of IGF2BP3 in Stomach Adenocarcinoma included mutation and amplification.

Genes related to *IGF2BP3*

We used LinkedOmics to identify genes related with IGF2BP3 in stomach cancer. A volcano plot revealed that genes correlated with *IGF2BP3* expression (false discovery rate < 0.05; Fig. 5a). Heat maps showing genes that were positively and negatively correlated with *IGF2BP3* in stomach adenocarcinoma (TOP 50) (Fig. 5b). *PLAGL2*, *GET4*, *IGF2BP1*, *HMGA2*, *CLDN6*, and *HOXC13* positively correlated with *IGF2BP3* expression in stomach adenocarcinoma. *SMARCA2*, *TMEM66*, *CIRBP*, *NFIX*, *SLC25A12*, and *CYB5D2* negatively correlated with *IGF2BP3* expression in stomach adenocarcinoma (Fig. 5c).

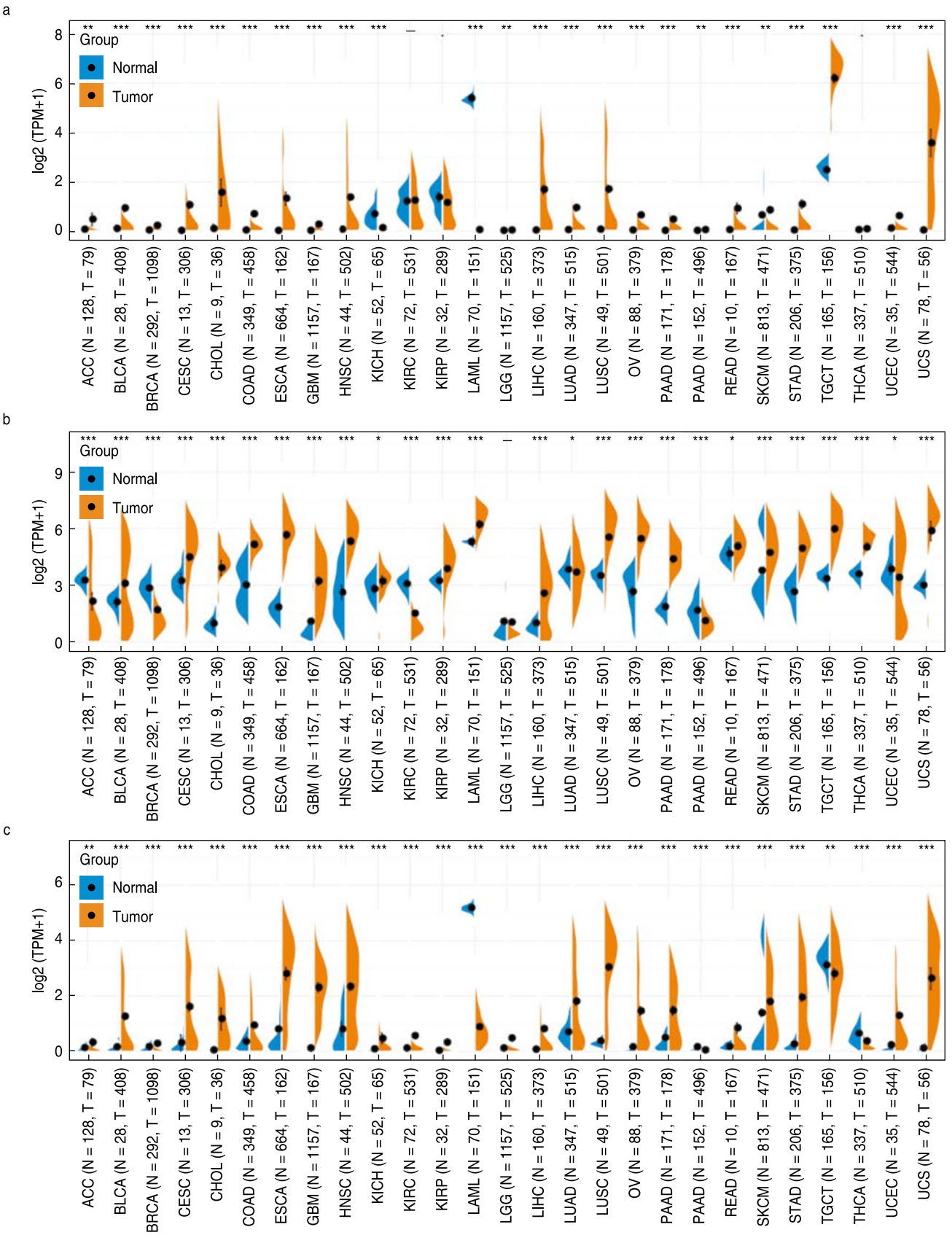

Fig. 1 Expression level of IGF2BP genes in gastric cancer
The expression of IGF2BP1 (a), IGF2BP2 (b), and IGF2BP3 (c) in tumors, analyzed using Sangerbox.

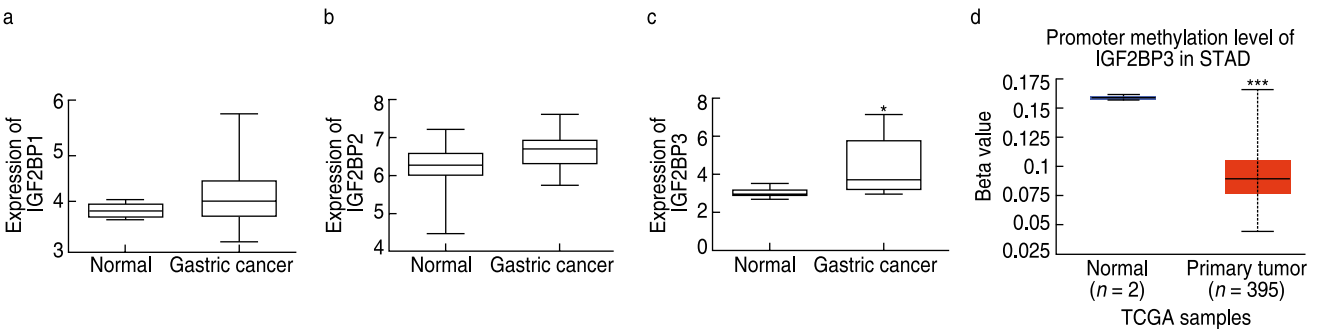

Fig. 2 *IGF2BP* gene analysis in gastric cancer, promoter methylation analysis. (a–c) Expression analysis of *IGF2BP1*, *IGF2BP2*, and *IGF2BP3* in paired gastric cancer and normal tissues in the GSE118897 dataset; (d) Promoter methylation level of *IGF2BP3* in gastric cancer. * $P < 0.05$.

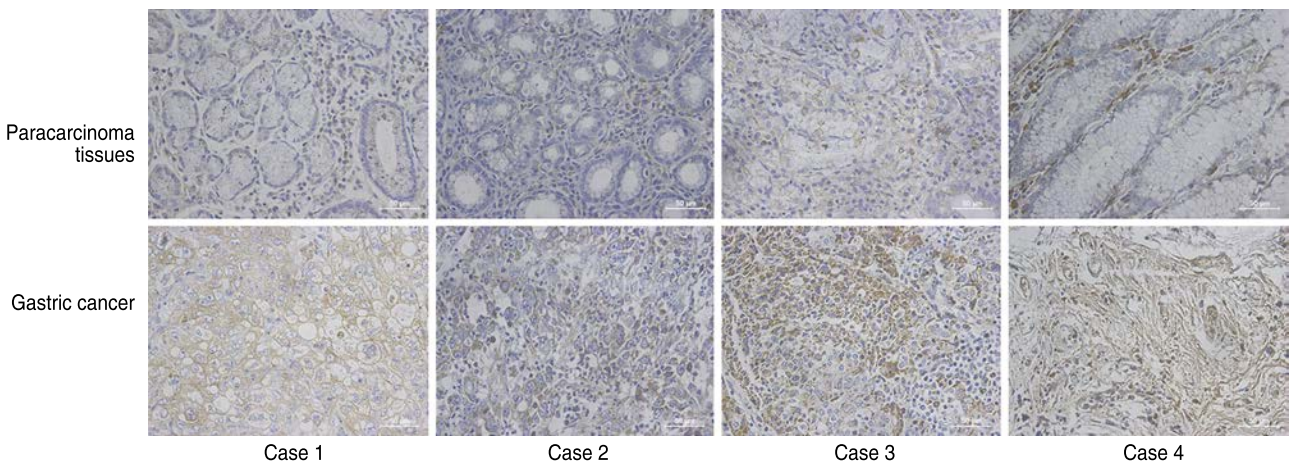

Fig. 3 Expression of *IGF2BP3* is higher in gastric cancer tissues.

Discussion

GC is a complex multifactorial disease, and genetic factors play a significant role in its development. Exploring the genes and signaling pathways related to the progression of GC could improve the early diagnosis rate and treatment options.

Recently, intensive research has demonstrated that IGF2BP3 is abnormally expressed in various tumor types, including human gliomas [10], neuroendocrine tumors of the lung [11], intrahepatic cholangiocarcinoma [12], prostate cancer [13], pediatric pilocytic and pilomyxoid astrocytoma [14], and endometrial clear cell carcinoma [15]. Zhou *et al.* [16]showed that *IGF2BP3* was dramatically overexpressed in GC tissues compared with normal gastric tissues, and higher expression of *IGF2BP3* was related to poor disease-specific survival. Collectively, these studies indicate that *IGF2BP3* may play a significant role in cancer. Therefore, assessing the expression of *IGF2BP3* and its interaction network may be useful for the diagnosis and treatment of cancer.

In recent years, the rapid development of bioinformatics methods that integrate big data has enabled advances in basic tumor research. In this study, we used bioinformatics tools to analyze the expression of *IGF2BP* genes and found that *IGF2BP3* was overexpressed in GC. We also found that the level of *IGF2BP3* promoter methylation in stomach cancer was significantly lower than that in control tissues, its elevated expression may be related to promoter hypomethylation. Additionally, heat maps showing genes positively and negatively correlated with IGF2BP3 expression in stomach adenocarcinoma. LinkedOmics showed that *PLAGL2*, *GET4*, *IGF2BP1*, *HMGA2*, *CLDN6*, and *HOXC13* were the top genes positively correlated with IGF2BP3 expression in stomach adenocarcinoma. PLAGL2 (pleomorphic adenoma gene like-2), a zinc finger PLAG transcription factor, is active in cancer progression. PLAGL2 could promote cell proliferation, migration and invasion in gastric cancer, play an important role in the stabilization of Snail1, and affect the Snail1-mediated GC cell proliferation and migration [17]. A previous study showed that *GET4* is one of the

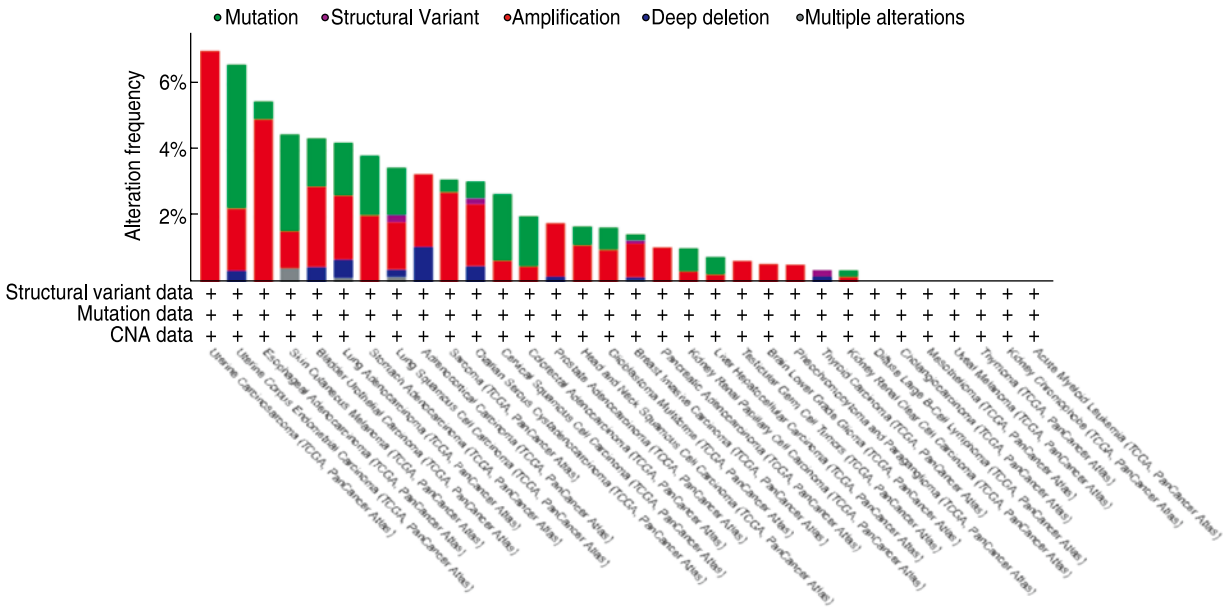

Fig. 4 Genetic alteration characteristics of IGF2BP3

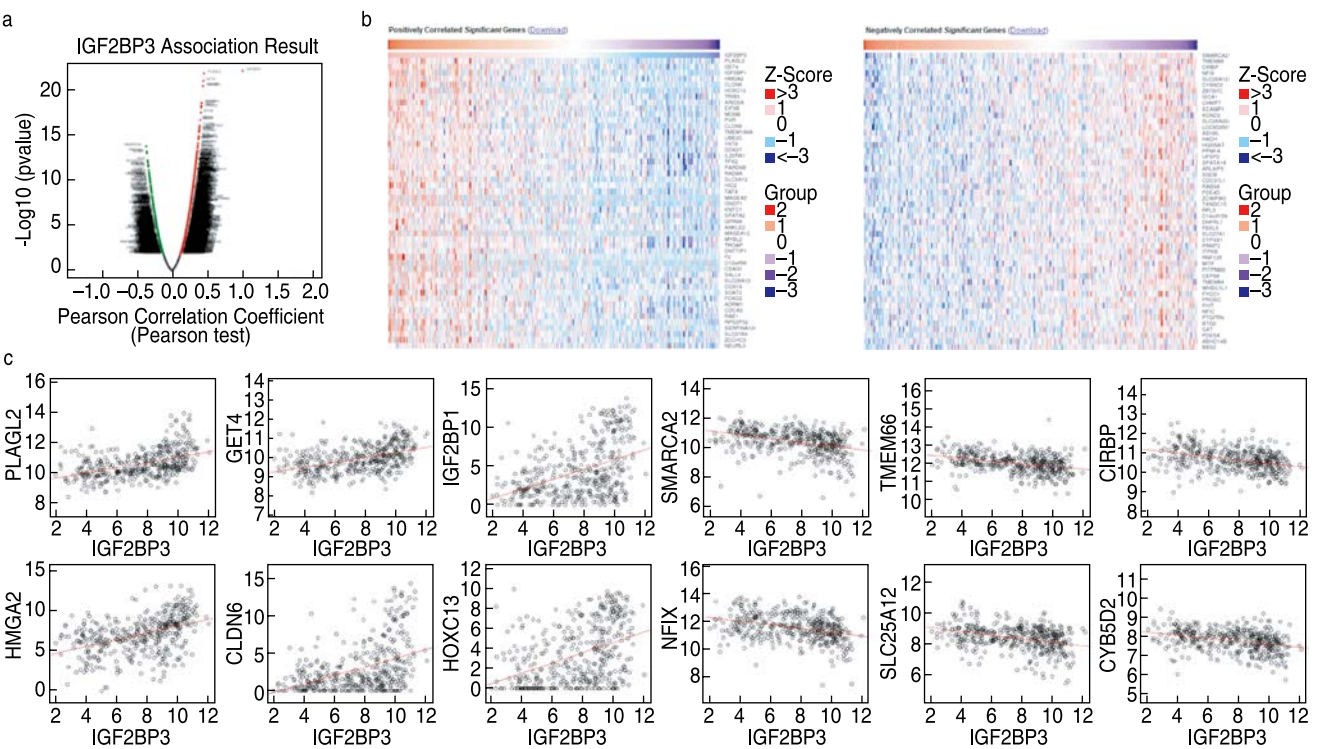

Fig. 5 Genes related to *IGF2BP3* in gastric cancer

(a) Pearson's test was performed to calculate correlations between IGF2BP3 expression and genes in gastric cancer by LinkedOmics; (b) Heat maps showing genes positively and negatively correlated with IGF2BP3 expression in stomach adenocarcinoma by LinkedOmics (TOP 50); (c) *PLAGL2*, *GET4*, *IGF2BP1*, *HMGA2*, *CLDN6*, *HOXC13*, *SMARCA2*, *TMEM66*, *CIRBP*, *NFIX*, *SLC25A12*, and *CYB5D2* were correlated with *IGF2BP3* in stomach adenocarcinoma.

genes altered in intestinal- and diffuse-type GC that were analyzed in chromosomal instability and genomically stable samples in TCGA RNA-seq data [18]. IGF2BP1 is involved in regulatory processes of long noncoding

(lnc)RNAs in GC cancer; lncRNA TRPM2-AS acted as a microRNA sponge for miR-612 and miR-612 could target IGF2BP1. Silencing the expression of IGF2BP1 inhibited GC cell proliferation and induced GC cell apoptosis; these findings reveal that IGF2BP1 has an oncogenic function in GC [19]. LncRNA GLCC1 regulates the migration and invasion of GC cells by enhancing the interaction of c-Myc/IGF2BP1 [20]. The high mobility group AT-hook 2 (HMGA2) is implicated in gastric carcinogenesis. The expression of HMGA2 significantly increased in GC samples compared with that in adenoma and normal gastric tissues. Multivariate analysis predicted that the expression of HMGA2 protein may be a useful prognostic marker for tumor recurrence [21]. Overexpression of HMGA2 induced GC cell sphere formation and migration [22]. Claudin6 (CLDN6) is a member of the tight junction family that participates in signal modulation in cancers [23]. CLDN6 expression was upregulated in both GC cell lines and tissues, and CLDN6 promoted GC proliferation and invasive ability [24]. *CLDN6* acts as a GC-promoting gene and may be a possible prognostic marker [25]. Analysis of the differentially expressed mRNAs and lncRNAs in 375 gastric adenocarcinomas and 32 adjacent non-tumor tissues on TCGA showed that lncRNA HOXC-AS3 may be a potential biomarker for gastric adenocarcinoma. HOXC-AS3 may regulate various *HOX* genes, including *HOXC13* in gastric adenocarcinoma [26]. LinkedOmics showed that *SMARCA2*, *TMEM66*, *CIRBP*, *NFIX*, *SLC25A12*, and *CYB5D2* were the top genes negatively correlated with IGF2BP3 expression in stomach adenocarcinoma. *SMARCA2* is a chromatin remodeling gene that plays vital roles in oncogenesis [27]. Somatic mutations of SMARCA2 have been reported in GC. Depletion of SMARCA2 in GC cell lines promoted cell proliferation [28]. TMEM66 may be related with multiple sclerosis and is likely a promising biomarker for multiple sclerosis [29]; nevertheless, the function of TMEM66 in cancer needs further study. CIRBP is a cold-shock protein, involved in cancers and inflammatory diseases, that regulates target mRNA. *CIRBP* is primarily thought to act as an oncogene, although it may also play a role in tumor suppression [30]. CIRBP is overexpressed in both bladder cancer tissues and cell lines, and can promote the proliferation and migration of bladder cancer cells [31]. CIRBP expression is higher in pancreatic ductal adenocarcinoma tumor tissues than in corresponding paracarcinoma tissues. CIRBP knockdown suppressed the proliferation of PANC-1 and SW1990 cancer cells, and overexpression of CIRBP promoted the proliferation of PANC-1 and SW1990 cells [32]. The role of CIRBP in gastric cancer requires further investigation. NFIX is a member of the nuclear factor I (NFI) family, which plays an important role in the development of several organs in mammals [33]. In GC, miR-625-5p targeted NFIX, and overexpression of NFIX could rescue the effect of LINC00511 silencing [34]. SLC25A12 (AGC1) is a vital component of the malate-aspartate shuttle, and SLC25A12 can affect pulmonary metastasis [35]. SLC25A12 was reactivated in HepG2 cells via CREB recruitment and histone acetylation. Silencing SLC25A12 inhibits the proliferation of HepG2 cells by regulating the cell cycle [36]. The expression of SLC25A12 is aberrant in acute myeloid leukemia (AML); it is overexpressed in AML patients compared with healthy people, and the expression of SLC25A12 is related to shorter event-free survival and overall survival of AML patients. SLC25A12 is a potential prognostic biomarker for AML [37]. CYB5D2 suppresses the proliferation of MCF7 cells and is a potential tumor suppressor in breast cancer [38]. CYB5D2 inhibits the invasion of HeLa cells. The expression of CYB5D2 was reduced in cervical squamous cell carcinomas [39]. Some of these predicted genes, which may be related to IGF2BP3, have been reported to play essential roles in gastric cancer. Overall, our results may be a starting point for further research on the function of IGF2BP3 in GC.

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Conflicts of interest

The authors indicated no potential conflicts of interest.

Author contributions

All authors contributed to data acquisition. All authors reviewed and approved the final version of this manuscript.

Data availability statement

The data that support the findings of this study are available from Yulong Li .

Ethical approval

This study was approved by the Ethics Committee of Shanghai Outdo Biotech Company.

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