Decreased expression of Insulin-like growth factor binding protein 3 and its death receptor in association with poor prognosis of the patients with gastric cancer

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Abstract

Background: Gastric cancer (GC) as one of the most common cancers, serves as the third cause of cancer-related death. Insulin-like growth factor binding protein 3 (IGFBP-3) and its novel death receptor (IGFBP-3R) has been shown to have an anti-tumor effect however their prognostic values have not been elucidated yet.

Methods: IGFBP-3 and IGFBP-3R expression were evaluated with quantitative real-time polymerase chain reaction and western blotting in patients with GC (N=68). The relationship between prognostic factors such as tumor stage, tumor grade, tumor size, metastases, and Overall Survival (OS) with IGFBP-3/IGFBP-3R expression was also assessed.

Results: IGFBP-3 and its death receptor expression were determined as dialectic evidence. In the experimental procedure, IGFBP-3 and IGFBP-3R expression were reduced in tumor tissues significantly. We found that there was an association between the reduction of IGFBP-3 with lymph node metastasis and TMN staging ($P < 0.001$). In addition, IGFBP-3R expression was associated with tumor size ($P = 0.004$), lymph node metastasis ($P < 0.001$), differentiation ($P = 0.002$) and TNM classification ($P = 0.005$). Interestingly, we presented that the downregulation of IGFBP-3R was stage-dependent. In OS analysis, we offered that low levels of IGFBP-3R mRNA expression were unfavorable with survival rate ($P = 0.002$). IGFBP-3 regardless of its relationship with some prognostic parameters, there was no significant relationship between IGFBP-3 and OS.

Conclusion: The findings of this study showed that IGFBP-3R has the potential to be a new prognostic biomarker of GC. But despite some benefit of IGFBP-3, it cannot be accepted as a prognostic biomarker. However, this finding must be improved and confirmed by future studies.

Introduction

Gastric cancer (GC) occupies the fifth most common and the third position for the leading cause of cancer-related deaths worldwide. The 5-year survivals in gastric cancer patients with adjuvant treatment can extend to be less than 23%, making this type of cancer as one of the most lethal cancers (1, 2). GC treatment is more likely to be a combination of chemotherapy, radiation and surgery. Mortality of GC was decreased during the past decades, resulting from surgical achievements and adjuvant therapy. GC identification usually takes place just after its symptoms represented in its advanced stage (3). Due to the poor prognosis, understanding of the molecular mechanism involved in cancer progression can improve prognosis and prevent metastasis to other organs (4, 5).

Insulin-like growth factor-binding protein 3 (IGFBP-3) is known as a multi-functional protein which can bind to 80% of IGF-I and II with high affinity to modulate their bioactivity (6). This conserved protein enables to form a ternary complex with acid-labile subunit (ALS) increasing IGF-I/II half-life (7). The main function of IGFBP-3 was identified in a wide variety of cancers.

IGFBP-3 has also been shown to induce apoptosis in an IGF-independent manner (8). For the first time, Oh Y demonstrated that IGFBP-3 can bind to the specific receptor in IGF-independent action (9). IGFBP-3 receptor (IGFBP-3R), a single span membrane protein, was identified as a novel cell death receptor by Ingerman. In addition, it was also demonstrated that IGFBP-3R which is widely expressed in most of the tissues and encoded by TMEM 219 (Transmembrane protein 219) gene, contains 240 amino acids residues in length and interacts with IGFBP-3 in the cellular membrane (10). Recently it has been shown that impairing of IGFBP-3/IGFBP-3R axis occurs in many of malignancies. IGFBP-3 expression appears to be suppressed in many cancers, due to some epigenetic alteration like hypermethylation (11). In addition, IGFBP-3R expression is significantly reduced in invasive breast ductal carcinoma, pancreatic ductal adenocarcinoma, and prostate tumor cells (10, 12). Thus, investigation of IGFBP-3/IGFBP-3R axis may provide prognostic and therapeutic value for primary diagnosis/staging and gastric cancer treatment.

In the present study, IGFBP-3 and IGFBP-3R expression were evaluated using qRT-PCR and western blotting in GC tumor tissues in compared with their normal adjacent tissues to serve as the potential prognostic marker in GC. The association of mentioned protein expression with clinicopathological features was evaluated as well.

Materials And Methods

RNA sequencing data Analysis

In order to determine the level of expression of TMEM 219 (IGFBP-3R) and IGFBP-3, four RNA-Seq datasets (SRP135952, SRP133891, SRP012016, SRP073446) from the Sequence Read Archive (SRA) (www.ncbi.nlm.nih.gov/geo) and three datasets (GSE122401, GSE63288, GSE106338) from Gene Expression Omnibus (GEO), all related to patients with GC, were downloaded. The datasets from SRA was analyzed to process raw data by using Ubuntu (18.04 LTS) and then was statistically interpreted to determine Differential Expression Genes (DEGs) by using software R (version 3.5.1). According to the low-quality sequence, the sequences were trimmed using Trimmomatic to remove reads with quality less than Q20 and adapter (first 15 bp of reads) (13). High-quality data (All clean reads) were mapped to Homo sapiens genome reference (UCSC version hg38) using HISAT2 (14) with mapping efficiencies around 97%, the counting was performed using HTSeq followed by differential expression analysis on each normal and tumor gastric replicates (15). In the end, normalization of the count was performed using R/Bioconductor limma package. All Geo dataset were already normalized and just the analysis of differential expression was performed using limma to determine DEGs (16).

Clinical Tissue Samples

All of 68 pair gastric cancer samples collected from gastric cancer surgical specimens, between April 2014 until September 2016 from Iran National Tumor Bank (INTB, Tehran, Iran). Each patient was written informed consent form, procedures according with the ethical standards of the institutional and/or national research committee of the 2013 Helsinki declaration and investigation has been approved with ethical committee members of the Medical University of Isfahan (Ethic number: 396386). Specimens

without prior radiotherapy, chemotherapy or any treatments were enrolled in the study. All of the samples were evaluated by two independent pathologists blinded to the clinical features. Samples characterized according to the American Cancer Society and tumor-node-metastasis (TNM) classification system guidelines (17). Normal samples were removed from the marginal zone of cancer tissue and used as a control. Immediately, all samples were snap-frozen in liquid nitrogen until the relevant assays were performed. The clinicopathological features of samples were summarized in Table 1.

Table 1

Association of clinicopathological features with IGFBP-3 and IGFB-3R mRNA relative expression in 68 patients with gastric cancer. * ($P < 0.05$) indicates that the differences have statistical significance.

*Pearson chi-square tests.

Parameters	Number of patients	IGFBP-3 ¹ mRNA expression		P value ²	IGFBP-3R ³ mRNA expression		P value ²
		Low	High		Low	High	
Age				0.250			0.163
< 61	28 (41.2%)	12	16		12	16	
≥ 61	40 (58.2%)	28	12		24	16	
Sex				0.536			0.254
Male	54 (79.4%)	32	22		30	24	
Female	14 (20.6%)	8	6		6	8	
Tumor size				0.146			0.004
< 6	34 (50%)	14	20		12	22	
≥ 6	34 (50%)	26	8		24	10	
Lymph node invasion				< 0.001			< 0.001
Positive	50 (73.5%)	34	16		32	18	
Negative	18 (26.5%)	6	12		4	14	
Differentiation				0.186			0.002
Poor	18 (26.5%)	8	10		0	18	
Moderate	40 (58.8%)	26	14		30	10	
High	10 (14.7%)	6	4		6	4	
TMN stage				< 0.001			0.005
IB + II	26 (38.2%)	10	16		10	16	

¹ Insulin-like growth factor binding protein-3

² P-value was calculated from one-way ANOVA with Tukey post hoc and independent T-test

³ Insulin-like growth factor binding protein-3 receptor

Parameters	Number of patients	IGFBP-3 ¹ mRNA expression		P value ²	IGFBP-3R ³ mRNA expression		P value ²
		Low	High		Low	High	
IIIA	18 (26.5%)	10	8		8	10	
IIIB + IV	24 (35.5%)	20	4		18	6	
¹ Insulin-like growth factor binding protein-3							
² P-value was calculated from one-way ANOVA with Tukey post hoc and independent T-test							
³ Insulin-like growth factor binding protein-3 receptor							

Chemicals, Reagents, And Antibodies

RNA extraction reagent (RXNTM-plus) and DNase I was provided from Cinnagen (Cinnagen, Tehran, Iran). cDNA synthesis kit and the enhanced chemiluminescent detection system (ECL) were purchased from Takara (Tokyo, Japan). All primers and qPCR Master Mixmix SYBR Green (high ROX) obtained from Ampliqun (Herlev, Denmark). Primary sheep polyclonal anti-IGFBP-3R (AF7556-SP) and secondary donkey anti-sheep IgG horseradish peroxidase (HRP)-conjugated antibody (HAF016) were purchased from R&D (Minneapolis, USA). Mouse monoclonal anti-β-actin and anti-IGFBP-3, secondary mouse anti-goat IgG HRP-conjugated was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Electrophoresis reagents and materials provided by Bio-Rad (Hercules, CA, USA). Other chemical and reagent obtained from Sigma Aldrich (St. Louis, MO, USA).

Quantitative RT-PCR

Before RNA extraction, tissues were washed with ice-cold phosphate buffer saline (PBS). Total RNA was extracted from all tissues by using RXNTM-plus according to the manufacturer's protocol. Frozen tissues (20–30 mg) homogenized by the bead-milling method in 1 ml of RXNTM-plus reagent as described previously(18). Subsequently, for the elimination of genomic DNA, the suspension was treated with DNase I. RNA concentration was then determined by ultraviolet spectrophotometer (BioTek, Winooski, VT, USA) using A260/A280 ratio and then the RNA samples were stored at -80 °C.

Total RNA (2 µg) was used for cDNA synthesise according to the kit's protocol as following: reaction mixture up to 10 µl and without a hot start, 1 cycle at 37 °C for 15 min for reverse transcription and 94 °C for 30 s for enzyme inactivation. Real-time PCR was performed utilizing an ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA). The sequences of primers were used as follow: IGFBP-3 forward: 5'-GGTGTCTGATCCCAAGTTCC-3', IGFBP-3 reverse: 5'-ACCATATTCTGTCTCCCGCT-3'; IGFBP-3R forward: 5'-TGACCACCTTGAACCTTCG-3', IGFBP-3R reverse: 5'-

GCAGAAGATCCTTTCAATC-3'; GAPDH forward: 5'-CAGCCTCAAGATCATCAGC-3', GAPDH reverse: 5'-GGCAGTGATGGCATGGACT-3'.

The final volume of the reaction mixture (10 µl) contained 1 ng of cDNA template, 200 nM each of sense and antisense primers and 5 µl of 2X SYBR Green PCR. The reaction conditions were as follows: after an initial hot start (95 °C) for 10 min, amplification was performed for 40 cycles containing denaturation for 10 s at 94 °C, annealing for 30 s at 50 °C, and extension for 40 s at 72 °C. The amplification kinetics was recorded as sigmoid progress curves for which fluorescence was interoperated against the number of amplification cycles. The threshold cycle number was used to define the initial amount of each template. Fluorescence readings were carried out in every amplification cycle, using StepOnePlus (Applied Biosystems, Foster City, CA, USA). All measurements were performed in triplicate. The sizes of the amplified fragments were confirmed by agarose gel 2% electrophoresis.

The relative expression was normalized with the GAPDH as an internal control. Then fold change calculated according to the $2^{-\Delta\Delta C_t}$ method: $\Delta\Delta C_t = [C_{t \text{ gene of interest}} - C_{t \text{ GAPDH}}]_{(\text{cancerous tissues})} - [C_{t \text{ gene of interest}} - C_{t \text{ GAPDH}}]_{(\text{normal-adjacent tissue})}$ similar with our previous study

Western Blotting

IGFBP-3 and IGFBP-3R protein expression were evaluated with western blotting as described previously(12). Summary, 100 milligrams of the fresh frozen tissues washed three times with ice-cold PBS. The tissues were homogenized by the bead-milling method in 1 ml ice-cold RIPA buffer (50 mM Tris-Cl [pH 7.4], 150 mM NaCl, 15 mM $\text{Na}_4\text{P}_2\text{O}_7$, 20 mM NaF, 1 mM EDTA, 1 mM PMSF, 6 mM EGTA, 100 mM glycerol 3-phosphate, 1% NP-40 [NP-4] and 1% Sodium Deoxycholic acid supplemented with 0.5% freshly protease and phosphatase inhibitors cocktail (Melford). The lysates were harvested with centrifugation (14,000 rpm) at 4°C for 25 min and the supernatant stored in -80°C.

The protein concentrations were measured with the Bradford protein assay. All protein samples were incubated with Laemmli buffer at 100 °C for 5 min, an equal amount (40 µg) of total proteins were separated by electrophoresis in a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Ready Gel, Bio-Rad). Following, transferred onto a polyvinylidene fluoride membrane (PVDF, Amersham Pharmacia Biotech), membranes were blocked with Tris-buffered saline, containing 5% non-fat dried milk and 0.1% Tween (TBS-T) for 2 h in room temperature. After 3 time wash with TBS-T, membranes incubated overnight with primary antibodies at 4 °C (1:1000 in TBS-T, 0.1% Tween 20 and 0.1% BSA). After washing three times with TBS-T for 5 min, the membranes were incubated in secondary antibodies (1:2500 in TBS-T, 0.1% Tween 20 and 0.1% BSA) horseradish peroxidase (HRP). After three times washing with TBS-T protein bands were detected with ECL reagent. All bands were normalized by β -actin as the internal control. The relative intensity of all bands was quantified by densitometry, using the Image J software (NIH, Bethesda, MA, USA).

Statistical analysis

Datasets from SRA database was analyzed to process raw data using Ubuntu (18.04 LTS) and then, to statistical calculation and interpretation of Differential Expression Genes (DEGs) used Statistical software R (version 3.5.1, <https://www.r-project.org/>). The comparison of RNA and protein relative expression levels between the normal and the tumor tissues assessed with paired Student's t-test. The One-way ANOVA (with Tukey posthoc) and independent sample T-test were used to evaluate the relationship between clinicopathological parameters and IGFBP-3/IGFBP-3R expression. The overall survival rates were calculated by the Kaplan–Meier method and differences in survival rates for between subgroup patients (high and low expression) were analyzed with the log-rank test. All experiments performed as triplicate and data were presented as mean $\pm$ SEM. Statistical significance was determined at the level of $P < 0.05$. All data were analyzed with SPSS 22 (SPSS, Chicago, IL, USA).

Result

Patients

This study contains 54 males and 14 females. Median ages of patients with GC were 62 ± 10 years (ranged 33–76 years). The tumor size was classified into two groups based on the mean (6 centimeters), there are three grades for tumors categorized to poor, moderate and well differentiation (Grade 3, 2 and 1, respectively) the stage was classified in IB + II, IIIA, and IIIB + IV. All clinicopathological features of patients were summarized in Table 1.

Identifying the IGFBP-3 and its death receptor gene expression in databases

After filtering the raw count of gene expression matrix that is lowly expressed (30% of genes in each dataset had zero counts across all samples), we checked the distribution of the raw read counts after log2 transformation using boxplot (Fig. 1a). Read count normalization of CPM (counts per million) was performed on gene expressing matrix with limma (Fig. 1b) and after normalization, limma was also used to screen the DEGs between gastric tumor and normal groups with a special threshold ($\text{padj} < 0.05$, $\log_2 \text{FC} < -1$ | $\log_2 \text{FC} > 1$) in all datasets. The significance level of the expression of TMEM 219 and IGFBP-3 in each dataset is shown in the Table 2. In most datasets, IGFBP-3 was down-regulated in tumor tissue and in two datasets reduction in the expression was significant. According to the results, TMEM 219 showed controversial expression changes in tumor tissues and paired normal tissues so needed to be further analyzed using other methods like qRT-PCR and western blotting.

Table 2

Gene expression level of IGFBP-3 and TMEM 219 (IGFBP-3R) in different RNA-seq datasets of gastric cancer (normal vs tumor) with a special threshold ($p_{adj} < 0.05$, $\log_2 FC < -1$ | $\log_2 FC > 1$). These two genes were differentially expressed In GSE122401, GSE63288 and GSE106338. The level of gene expression of these two gens in four data sets (SRP135952, SRP133891, SRP073446, SRP012016) was low but Significance level of genes expression was not impressive.

Dataset	Number of samples (tumor/normal)	Gene symbol	log2FC	p _{adj}	Gene regulation (p _{adj} < 0.05, log ₂ FC < -1 log ₂ FC > 1)
GSE122401	160 (80/80)	TMEM219	5.929084744	4.30E-06	Up-regulated
GSE63288	44 (22/22)	IGFBP-3	-4.029501	0.000230573	Down-regulated
		TMEM219	0.038779382	0.886275834	-
GSE106338	6 (10/5)	IGFBP-3	-0.644756605	0.015192113	Down-regulated
		TMEM219	0.304485256	0.040597407	-
SRP133891	6 (3/3)	IGFBP-3	6.390124314	2.69E-13	UP-regulated
		TMEM219	-0.024690081	0.999295723	-
SRP073446	6 (2/3)	IGFBP-3	-1.214712018	0.477523199	-
		TMEM219	1.709564312	0.034660516	-
SRP135952	12 (6/6)	IGFBP-3	0.064407026	0.961976295	-
		IGFBP-3	-2.08302277903425	0.99993861	-
SRP012016	6 (3/3)	TMEM219	0.358402202	0.99993861	-
		IGFBP-3	-1.751039396	0.128781382	-

IGFBP-3 expression reduced in GC tumor tissue in comparison with normal adjacent tissue

We measured the IGFBP-3 expression in 68 paired GC tissue and normal marginal zone. In real-time qRT-PCR, the results indicated that the mRNA relative expression of IGFBP-3 markedly reduced in cancerous tissue in comparison with normal adjacent tissue (Fold change: 0.47 ± 0.04 , Fig. 2a and $P < 0.001$).

Analyzing based on different TNM stage and fold changes indicated that overall IGFBP-3 expression decreased in all stages (Fig. 2b). Also, relative protein expression of IGFBP-3 evaluated with western blotting by using β -actin as an internal control (Fig. 3a). The results indicating that the protein expression was reduced compared with paired normal tissue (Fold change: 0.85 ± 0.13 , Fig. 3b and $P < 0.001$). Then, the pattern of protein expression for IGFBP-3 was analyzed. In according to TNM staging, IGFBP-3 protein reduction in stage IB + II stage was not significant, but in IIIA (0.62 ± 0.11 , $P = 0.018$) and IIIB + IV stages (0.45 ± 0.05 $P < 0.001$) were statistically significant (Fig. 3c).

IGFBP-3R expression reduced significantly in GC tumor tissue compared with normal adjacent tissue

Our findings indicated that mRNA relative expression of IGFBP-3R was markedly reduced in GC tumor tissue in comparison to paired normal tissue (Fold change: 0.49 ± 0.05 , Fig. 4a and $P < 0.001$). TNM stage analysis revealed that IGFBP-3R mRNA expression were significantly reduce stage dependent in IB + II (0.69 ± 0.12 , $P = 0.003$), IIIA (0.56 ± 0.11 , $P < 0.001$) and IIIB + IV (0.22 ± 0.06 , $P < 0.001$) stage (Fig. 4b). In western blotting analysis (Fig. 5a), IGFBP-3R protein expression normalized by β - actin, was significantly reduced in GC tumor tissue (Fold change: 0.53 ± 0.02 , Fig. 5b and $P < 0.001$). In addition, analysis of IGFBP-3R protein expression pattern indicated that it depends on stages IB + II (0.77 ± 0.01 , $P < 0.001$) and IIIA (0.37 ± 0.02 , $P < 0.001$). But decreasing expression of this protein in not significant in IIIB + IV stage (Fig. 5c).

Association between IGFBP-3 and IGFBP-3R expression with clinical-pathological parameters in patients with gastric cancer

The relationship between IGFBP-3 and IGFBP-3R relative expression with clinicopathological variables were analyzed by One-way ANOVA (with Tukey posthoc) and independent sample T-test and listed in Table 1. The analysis revealed that IGFBP-3 expression associated with lymph node invasion and TNM stage ($P < 0.001$) strongly. Moreover, analyzing IGFBP-3R expression revealed that the low-level expression associated with tumor size ($P = 0.004$), lymph node invasion ($P < 0.001$), differentiation ($P = 0.002$) and TNM stage ($P = 0.005$). However there was no relationship between IGFBP-3 and IGFBP-3R expression with other parameters including age and sex.

Correlation of the IGFBP-3 and its death receptor expression level with overall survival

The association of the Overall Survival (OS) of the GC patients ($N = 68$) with IGFBP-3 and its death receptor expression were evaluated. The analysis was performed as Kaplan-Meier with the log-rank test. The low or high expression level was interpreted according to the mean. Patient with high levels of IGFBP-3R mRNA expression ($N = 32$) had better survival in comparison with low levels of mRNA expression ($N = 36$) (survival time: 24 ± 1.31 months versus 18 ± 6.33 months, $P = 0.002$, Fig. 6a). However, survival rate analysis based on IGFBP-3 indicated no significant difference between the low and high expression of IGFBP-3 ($P = 0.075$, Fig. 6b).

Discussion

Proteomics study around the protein biomarkers has indicated that some proteins might serve as the potential prognostic biomarker (19–21). It has been revealed that IGFBPs, specifically IGFBP-3, could be considered as a potential prognostic biomarker (22–24). In the present study, we provide a new information around IGFBP-3/IGFBP-3R axis gene expression via RNA-seq analysis, mRNA and protein analysis as well as their correlations with clinicopathological features. First, we considered some available raw and processed RNA-seq datasets related to GC tumor and adjacent normal tissues. Then, we assessed the differential expression level of IGFBP-3 and its death receptor (TMEM 219). Our analysis of datasets revealed that IGFBP-3 is down-regulated while, the results for TMEM 219 expression were controversial (Fig. 1 and Table 2). Despite having a wide dynamic range of detection and negligible background signal in comparison with other known detection methods (25), it is believed that RNA-Seq data of differentially expressed genes (DEGs) might because of its variation and bias. Therefore proper normalization methods and validation with an accurate gold standard like qRT-PCR were also definitely needed (26, 27). This fact interested us to further consider the differential expression using more exacting conformational methods like qRT-PCR and western blotting analysis.

In the present study, we also showed that IGFBP-3 and IGFBP-3R expression is reduced in mRNA level (Fig. 2a and Fig. 4a). In line with our findings, it has been reported that IGFBP-3 promoter methylation, and its reduction of expression, in early-stages of GC is critical in predicting survival (28). Zhang et al also revealed that IGFBP-3 has a protective effect the development of GC and its down regulation affect the prognosis (29).

The exact mechanism highlighting IGFBP-3 and its receptor roles in GC progression are partially understood (7, 8, 12, 30). In this regard, IGFBP-3 has been extensively considered as a p53-inducible gene that insatiate apoptosis in cells and tumor (31). Baxter et al also declared that IGFBP-3 is known as a novel ligand mediating apoptosis through nucleus internalization (32, 33). Moreover, previous in silico study revealed that IGFBP-3 phosphorylation on ser111 which occurred in apoptosis induction, make a repulsive effect with IGF-I facilitating IGFBP-3 interaction with IGFBP-3R in outer membrane (34). Furthermore, Xue and collagus also indicated that IGFBP-3 can suppres some invasion factor like urokinase-type plasminogen activator (uPA) and MMP-14 (35). Harada and colleagues also showed that IGFBP-3 can induce apoptosis via IGFBP-3R in lung cancer (36). Similarly, IGFBP-3R interacts with nuclear factor kappa light chain enhancer of activated B cells (NF- κ B) pathway and suppresses tumor growth (37, 38). It was demonstrated that in cancer status, the increasing level of intra or extracellular proteases like Matrix metallopeptidases (MMPs) and serine proteases may affect IGFBP-3 level. Kallikrein 11 in breast cancer can degrade IGFBP-3 and increase the bioactivity of IGFs (39). This fact is supported with our IGFBP-3 western blotting analysis as well (Fig. 3b and 3c).

In this study, we also showed that IGFBP-3 was markedly reduced in mRNA and protein level (Fig. 2a and Fig. 4a). This decline correlated with the stage and grade progressions (Table 1, Fig. 2b and 3c). To support IGFBP-3 roles in a higher stage of cancer, a meta-analysis indicated that the reduced IGFBP-3 expression is associated to higher cancer risk, lower survival rate, and more advanced tumor stages of esophageal cancer (46). Similarly, ovarian endometrioid carcinoma (EC), glioblastoma, colorectal cancer

and gastric adenocarcinoma have been reported to associate with low IGFBP-3 expression (22, 40–43). This fact can support our clinicopathological features analysis (Table 1). In line with the current study, the low IGFBP-3 expression has been reported to correlate clinically with higher invasion rate in different cancers including pancreatic ductal adenocarcinoma, ovarian carcinoma, prostate cancer, and Non-small cell lung cancer (NSCLC) (12, 40, 44, 45).

Similarly, a more recent study uncovered that low expression of IGFBP3 is linked to certain clinicopathological features and the poor overall survival of patients with hepatocellular carcinoma and pancreatic cancer (12, 47). However, in our study, IGFBP-3 expression was not correlated with survival, but IGFBP-3R expression was associated with poor survival (Fig. 6a and b).

Conclusion

Taken together the current study tried to highlight IGFBP-3/IGFBP-3R axis in GC and representing new information about IGFBP-3R. We demonstrated that a decrease in IGFBP-3 and IGFBP-3R expression is associated with clinicopathological features. Additionally, we demonstrated that IGFBP-3R relative expression was significantly associated with low survival time and poor prognosis in patients with GC. IGFBP-3 and its death receptor expression pattern indicated that they could be recruited as a potential biomarker for TNM staging and prognosis detection. However further investigations are needed for more validation.

Abbreviations

Gastric cancer (GC)

Insulin-like growth factor binding protein 3 (IGFBP-3)

Insulin-like growth factor binding protein 3 receptor (IGFBP-3R)

Overall Survival (OS)

Lymph Node Metastasis (TMN)

Acid-labile subunit (ALS)

Transmembrane protein 219 (TMEM 219)

Sequence Read Archive (SRA)

Gene Expression Omnibus (GEO)

Differential Expression Genes (DEGs)

Iran National Tumor Bank (INTB)

Horseradish Peroxidase (HRP)

Phosphate Buffer Saline (PBS)

plasminogen activator (uPA)

Matrix metalloproteinases (MMPs)

Endometrial carcinoma (EC)

Non-small cell lung cancer (NSCLC)

Declarations

Ethics approval and consent to participate

Human tissue experiments were reviewed and approved by the Isfahan University of Medical Sciences

Consent for publication

Not applicable

Availability of data and materials

All data and materials are available on request to corresponding author

Competing interests

All authors declare that there is no conflict of interest.

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Authors' contributions

A.A and contributed in the conception of the work, conducting the study, writing the draft.

A.Gh contributed in the conception of the work, approval of the final version of the manuscript, acquisition and analysis of data.

H.B contributed in the conception of the work or design of the work, acquisition and analysis of data and writing the draft.

A.E.R contributed in the conception of the work or design of the work, approved the final version of the manuscript

A.S contributed in the conception of the work or design of the work, acquisition and analysis of data and writhed the draft

M.R.M. supervised the study, contributed in the design of the work, acquisition and analysis of data revising the draft and approval of the final version of the manuscript

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Figures

Figure 2

Distribution of the raw read counts after log₂ transformation. a, Box plots of non-normalized count (log₂ (counts+1)) per sample (GSE122401). b, Box plots of normalized counts (logCPMs) per sample (GSE122401).

Figure 4

Down-regulation of the mRNA expression level of IGFBP-3 and TNM stage analysis in gastric cancer. Relative expression was performed with qRT-PCR and calculated with the $2^{-\Delta\Delta C_t}$ method, normalized all curve threshold with using GAPDH as an internal control. a, comparison of relative expression of IGFBP-3 in cancer tissue adjacent non-tumor tissues. b, analyzing of IGFBP-3 fold changes relative expression in gastric cancer stages. Each bar represents for fold change for stages and all stand shown as SEM. *** represented as $P < 0.001$ is significant vs. control group.

Figure 6

Protein expression of IGFBP-3 determined with western blotting. All bands normalized with β -actin as internal control, the intensity of all bands calculated with Image J software. a, a representative image of IGFBP-3 bands in tumoral cancer (T) and normal (N) tissue. b, ratio protein expression in gastric cancer tissue in comparison with normal tissue. c, analyzing of protein fold change according to TNM stage classification. All stand are shown as SEM, ** ($P < 0.01$) and *** ($P < 0.001$) is significant vs. control group. No Sig means not significant

Figure 8

Analyzing of IGFBP-3R mRNA expression fold change in gastric cancer. a, Comparison of IGFBP-3R relative expression gastric cancer tissue in comparison with normal adjacent tissue. b, analyzing of IGFBP-3R mRNA fold change according to stages. All stands are shown as SEM, ** ($P < 0.01$) and *** ($P < 0.001$) is significant according to the previous stage.

Figure 9

Analyzing of IGFBP-3R protein expression in gastric cancer and stages by using western blotting. All bands normalized with β -actin. a, representative bands in the tumor (T) and normal (N) tissue. b, IGFBP-3R protein expression reduced in all patient samples. c, analysis of IGFBP-3R pattern according to TNM stage classification. Each bar represented as fold change, all stands are shown as SEM, *** ($P < 0.001$) is significant vs. the previous stage and No Sig. means not significant.

Figure 11

Correlation of IGFBP-3 and IGFBP-3R expression with cumulative survival patient for 68 gastric cancer patient. Favored or non-favored patient with different expression (low and high expression based on mean) was calculated and analysis with the log-rank test. a, Low or high level of IGFBP-3R expression correlates with a poorer overall ($P=0.002$, log-rank test). b, relative expression of IGFBP-3 has no significant correlation with overall survival of a patient with gastric cancer.