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Research

Lower cytoplasmic expression of DDIT4 is associated with poor prognosis in gastric cancer patients

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Abstract

Introduction DNA damage-inducible transcript 4 (DDIT4), also known as Redd1, Dig2, and RTP801 was identified to be upregulated in response to a variety of cellular stresses, including DNA damage, endoplasmic reticulum stress, and energy stress. Several studies have discovered that dysregulation of DDIT4 involved in various cancers with paradoxical expression and roles. Hence, this study was designed to investigate the clinical significance and prognostic value of DDIT4 in different subtypes of gastric cancer (GC).

Materials and methods To evaluate the expression pattern of DDIT4 in GC tissues as well as adjacent normal tissue, we utilized immunohistochemistry on tissue microarray (TMA) slides.

Results Our findings revealed that nuclear expression of DDIT4 was higher in GC tissues than in non-malignant samples. Also, the cytoplasmic and membranous expression of DDIT4 were significantly lower in tumor samples ($P=0.007$ and $P=0.002$, respectively). The results indicated that there was a statistically significant association between low cytoplasmic and membranous expression of DDIT4 and advanced histological grade ($P=0.001$ and $P=0.016$). The survival analysis revealed that lowered cytoplasmic expression of DDIT4 is significantly associated with worse DSS ($P=0.038$).

Conclusion Lower cytoplasmic expression of DDIT4 could serve as a promising prognostic biomarker in GC.

Keywords Gastric cancer · DNA damage-inducible transcript 4 (DDIT4) · Immunohistochemistry (IHC) · Tissue microarrays (TMA) · Cytoplasmic expression

1 Introduction

Gastric cancer (GC) is the sixth most frequent cancer in the world and the third leading cause of cancer-related mortality [1].GC is frequently diagnosed at an advanced stage, and the prognosis is poor due to the metastasis, high recurrence rate, and drug resistance [2], which significantly limit the success of treatment modalities [3]. Prognostic

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biomarkers play essential roles in identifying benign tumors from malignant tumors, monitoring the progression of advanced GCs, and predicting survival outcomes [4–6].

The DDIT4 gene, also known as REDD1 or RTP801, is highly conserved from flies to humans [7]. DDIT4 was discovered in a screen for genes that are induced by hypoxia [8], and it was later discovered to be elevated in response to a number of other cellular stimuli, such as DNA damage, endoplasmic reticulum stress, energy stress, and glucocorticoid therapy [9–12]. The control of cell growth and division in response to cellular stress is essential for the survival of normal cells, whereas deficiencies in the stress response can promote cancer progression [13]. DDIT4 gene encodes a protein whose main function is to inhibit mTORC1 [11, 14, 15]. The DDIT4 inhibits mTORC1 by activating the upstream mTORC1 suppressors tuberous sclerosis complex 1 (TSC1) and tuberous sclerosis complex 2 (TSC2) [14, 15]. TSC1 and TSC2 proteins form a protein complex with GAP (GTPase-activating protein) activity that regulates RhebGTP levels. Rheb is a GTP-binding protein, and mTORC1 interacts with the G protein Rheb. It has been shown that GTP-loaded Rheb can activate mTORC1 in vitro. In normal condition, when growth factors are present, TSC2 is phosphorylated via an AKT-mediated pathway [16]. The association of phosphorylated TSC2 with 14-3-3 protein inhibits the function of TSC1/2, which increases Rheb-GTP and activates mTORC1 [17–19]. In response to hypoxia, the level of DDIT4 increased, and it was found to bind to the 14-3-3 protein. This resulted in TSC2/14-3-3 dissociation, which led to TSC1/2 activation, an increase in Rheb-GDP, and the inhibition of mTORC1. mTORC1 is a multiprotein complex and an important cell growth regulator [18, 19] (Fig. 1).

Recent research has demonstrated that DDIT4 dysregulation occurs in a variety of human cancers with contradictory roles. Several studies have linked DDIT4 to the tumor suppressor process through suppression of mTORC1 in breast cancer [20], colorectal cancer [21], sporadic clear cell renal cell carcinoma (ccRCC) [22], and non-small cell lung cancer [23].

As an oncogene, the overexpression of DDIT4 contributes to the inhibition of apoptotic processes and the stimulation of cancer cell proliferation, migration, and invasion [14, 24, 25]. This has previously been reported in GC [14], ovarian cancer (OC) [25], bladder urothelial carcinoma (BUC) [26] and ccRCC (patients with von Hippel Lindau- deficiency) [22].

In gastric epithelial cells, upregulation of DDIT4 has been found to increase cell proliferation, decrease apoptosis, and prevent S-phase arrest [14]. In addition, overexpression of the DDIT4 protein was observed to be related to decreased expression of pro-apoptotic proteins and higher levels of anti-apoptotic proteins in ovarian epithelial cells following RAS oncogene activation [15].

Increased DDIT4 expression has been identified as a prognostic factor for OC and BUC patients [25–27]. In addition, it has been reported that increased expression of DDIT4 mediates cancer therapy resistance in numerous tumors, such as brain, lung, and gastric, because it protects tumor cells from therapy [14, 28, 29].

In this study, we used immunohistochemistry (IHC) to determine DDIT4 expression and its subcellular localization (nucleus, cytoplasm, and plasma membrane) in a collection of 213 formalin-fixed, paraffin-embedded (FFPE) tissues

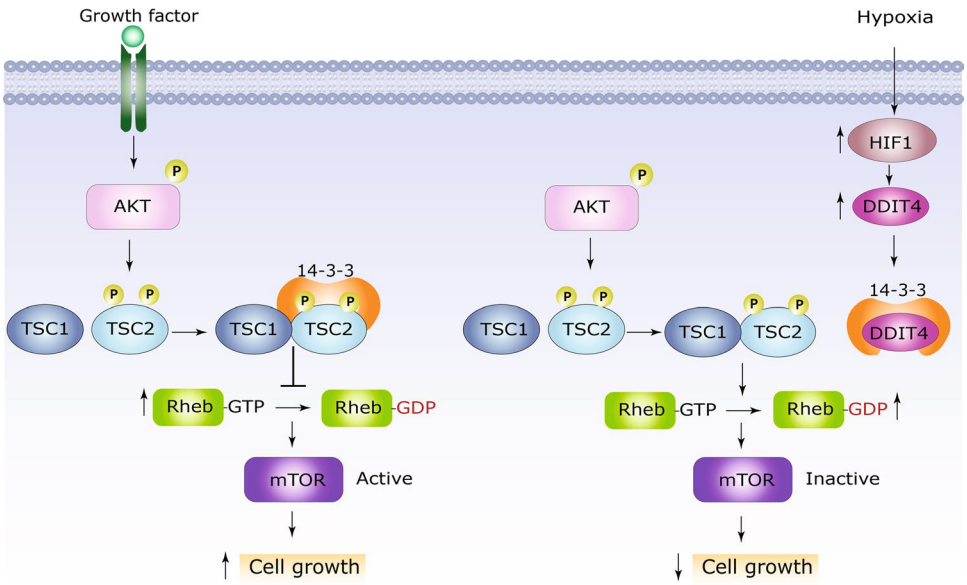

Fig. 1 The mTOR signaling pathway. In normal conditions (left), the growth factor activates AKT, which phosphorylates TSC2. This phosphorylation promotes the association of TSC2 with 14-3-3 protein, leading to formation of TSC1-pTSC2-(14-3-3) complex and mTOR activation via the GTP-binding protein Rheb (RHEB), resulting in increased cell growth. In contrast, under hypoxic conditions (right), increased expression of DDIT4 leads to formation of DDIT4 and 14-3-3 complex, therefor, the TSC1-pTSC2-(14-3-3) complex is not formed and leads to inactivation of mTOR signaling pathway

microarrays (TMAs) obtained from GC patients. Clinical and pathological characteristics, as well as patients' survival, were analyzed in relation to DDIT4 expression levels in various cellular compartments.

2 Materials and methods

2.1 Data mining about DDIT4 by bioinformatics tools

To investigate the RNA expression level of the DDIT4 gene in tumor and normal samples, we used the UALCAN database, which is based on "The Cancer Genome Atlas" (TCGA) [30]. We also used the STRING database to assess the potential protein–protein interaction (PPI) network of DDIT4. The PPI gene network was visualized using stringApp in Cytoscape software version 3.8.2 (confidence score 0.4) [31, 32]. To better understand the functions and biological pathways associated with these genes, we conducted enrichment analysis using the ClueGO plugin in Cytoscape [33]. This analysis included evaluations of molecular function, biological processes, and pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [34].

2.2 Patient's characteristics and tumor samples

A total of 213 FFPE tissues from GC tumor samples and 24 matched adjacent non-malignant tissues were collected from the Firoozgar, a major referral university-based in Tehran, Iran, during 2012–2020. DDIT4 expression was evaluated on all 213 samples. The patients with primary GC who had undergone gastrectomy surgery but had not received either chemotherapy or radiotherapy were included in the study. The GC tissue slides were stained using hematoxylin and eosin (H & E), and information on clinicopathological parameters including age, sex, histological subtype, tumor size, histological grade, primary tumor stage, tumor extension, vascular invasion, lymphovascular invasion, metastasis, and recurrence were also collected from medical records.

The under-study patients were followed for 114 months until February 2022. Disease-specific survival (DSS) was defined as the time between surgery and the date of cancer-related death. The interval between the primary operation and the last follow-up without any evidence of disease, tumor recurrence, or metastasis was determined as progression-free survival (PFS). This study received its ethical approval (Code: IR.IUMS.REC.1402.181) from the Research Ethics Committee of the Iran University of Medical Sciences.

2.3 Tissue microarray (TMA) construction

The construction of GC TMA blocks was performed as described previously [33]. Briefly, to prepare TMA blocks from each corresponding FFPE block, three most representative tumor areas were selected and marked after matching with H&E slides by two experienced pathologists (M.R. and Z.M.). Subsequently, the selected regions of blocks were punched (0.6 mm diameter) into TMA recipient blocks using Tissue Arrayer Minicore (ALPHELYS, Plaisir, France). According to great number of scientific publications, using a 0.6-mm-diameter punch for inserting into TMA recipient blocks is a well-established and approved method [36–38]. The majority of research prefers 0.6-mm punches, noting benefits like reduced damage to the donor block, preservation of more tissue, and the capacity to include greater number of cores in one recipient block [36]. According to TMA research, using two cores or more, increase accuracy by 95–99% and could solve the problem of heterogeneous antigen expression [39, 40]. In this study, three cores were punched from each sample and each core was scored individually, and the mean score calculated to represent the final score for each sample.

2.4 Immunohistochemistry (IHC) staining using DDIT4 antibodies

Briefly, after conventional dewaxation (60 °C for 30 min) and rehydrated with graded alcohol, endogenous peroxidase activity was blocked by 3% H_2O_2 for 20 min at room temperature, then slides were washed three times in Tris Buffered Saline (TBS). Antigen retrieval was performed in citrate buffer (pH 6.0) for 10 min in an autoclave. After washing, the slides were incubated with 5% sheep serum prepared in blocker protein (Dako, Denmark) for 20 min. Then, the sections were incubated with the polyclonal anti-DDIT4 antibody (ab71333, Abcam, USA, dilution: 1/80), overnight at 4 °C. In all experiment, non-immune rabbit IgG was used as isotype control. After three times washing, slides were incubated with secondary antibody (anti-rabbit/anti-mouse Envision (Dako, Glostrup, Denmark)) for 40 min at room temperature (RT).

Finally, the sections were colored by 3,3′-diaminobenzidine (DAB) (Dako, Denmark) substrate as a chromogen for 20 min, re-stained with hematoxylin (Dako, Denmark) for 5 min. Dehydration steps were conducted using serial dilutions of alcohol 70, 96, and 100%) and xylene and finally sealed with neutral gum for observation.

During the experiment, normal liver tissue were chosen to serve as positive controls for DDIT4. Images were captured using a digital sight DS-LS camera and H550S microscope (Nikon, Japan).

2.5 Evaluation of immunostaining

The expression levels of DDIT4 was semi-quantitatively evaluated, by two expert pathologists (M.R. and Z.M.), who were blinded to clinical and pathological parameters. DDIT4 expression patterns was evaluated by three methods of scoring, positive cell percentage, staining intensity, and histochemical score (H-score). The intensity of staining was divided into four groups: 0 (negative); 1 + (weak); 2 + (moderate); and 3 + (strong) staining. The percentage of positive cells was valued and scored semi-quantitatively from 0 to 100% and categorized according to: < 25% as 1, 25%–50% as 2, 51%–75% as 3, and > 75% as 4. Finally, for comparing all of the available data, H-score was also obtained by multiplying the intensity score (0–3) by the percentage scores (0–100%), which yielded the final scores from 0 to 300 [34]. The final H-scores were calculated from average of three score spots for each sample and were classified into two groups (low or high expression) based on the median DDIT4 expression level [5, 35].

2.6 Statistical analysis

All data were analyzed using the "Statistical software SPSS Version 25.0 (Armonk, NY: IBM Co). All the categorical data were reported by N (%), and quantitative data by mean with standard deviation (SD). Pearson's χ^2 and Spearman's correlation tests were performed to analyze the significance of the association and correlation between the expression of the DDIT4 proteins and clinicopathological features. Moreover, the comparisons between these groups were carried out by Kruskal–Wallis and Mann–Whitney U tests. The Kaplan–Meier method was used to plot survival curves with a 95% confidence interval (CI), and the log-rank test was used to compare survival outcomes between groups with low and high marker expression based on H score and intensity of staining and percentage of positive tumor cells. The variables that affected DSS or PFS were identified using the cox proportional hazards regression model. The statistically significant difference was defined in all analysis components as $P < 0.05$.

3 Results

3.1 Bioinformatics approaches

The results obtained from the TCGA database via UALCAN revealed a significant difference in RNA levels for DDIT4 gene between gastric cancer and normal tissues ($P = 0.002$) Fig. 2.

The PPI network construction based on STRING database indicated DDIT4 has a high confidence score with TP53 as a tumor suppressor in several tumor types and SFN (14-3-3) as shown Fig. 3A. Furthermore, the enrichment analysis of genes from the PPI network reveals their functional roles and biological significance, as detailed in Fig. 3B. This analysis indicated that DDIT4 is involved in important signaling pathways and biological processes, such as PI3K-AKT signaling, TOR signaling, and autophagy.

3.2 Patients' characteristics

The sample population in this study comprised a total of 213 GC patients, including two histological subtypes: 64 single ring cell carcinoma (SRC) and 149 Intestinal types. Among these gastric cancer patients, 158 were males (74.2%) and 55 were females (25.8%). The clinicopathological characteristics of the samples are summarized in Table 1.

3.3 Evaluation of DDIT4 expression in GC samples and their adjacent non-malignant tissues

The DDIT4 expression levels were assessed using the IHC method on TMA slides by three different scoring methods, comprising the intensity of staining, percentage of positive tumor cells, and H-score. DDIT4 was expressed at various

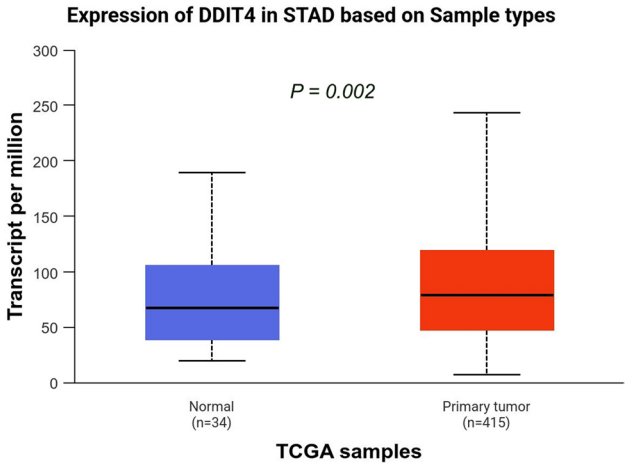

Fig. 2 Box Plot of TCGA results for DDIT4 RNA expression in the UALCAN database. The results revealed a significant difference in DDIT4 RNA expression between gastric cancer (STAD: Stomach adenocarcinoma) tissues and normal tissues (P = 0.002)

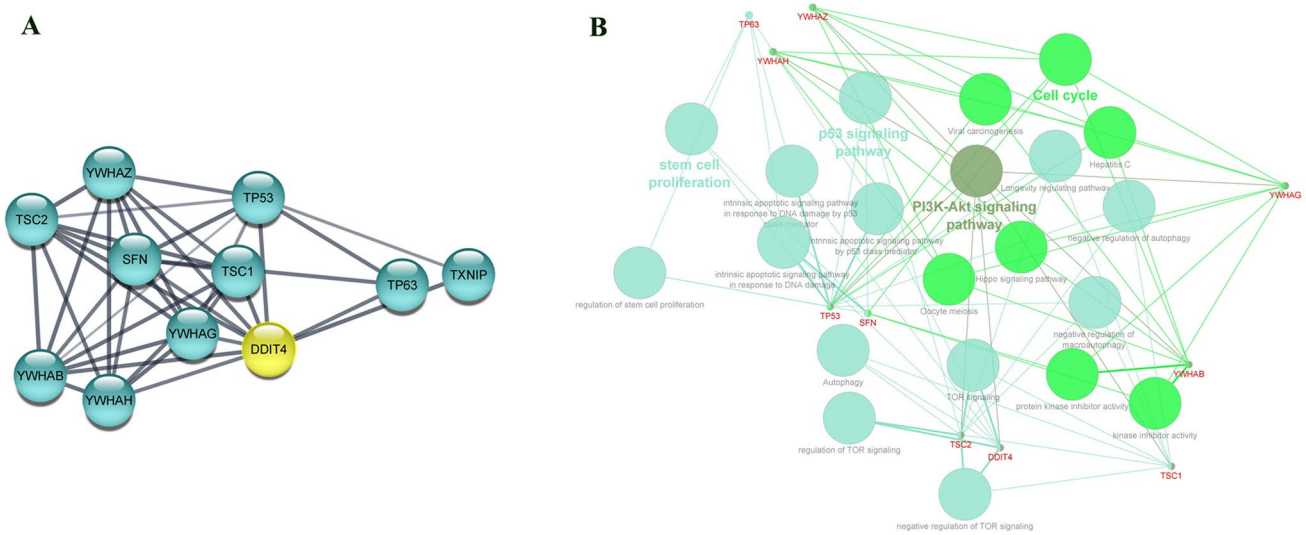

Fig. 3 Protein–Protein Interaction (PPI) Network and Enrichment Analysis. **A** PPI network for the DDIT4 protein was constructed using the StringApp plug-in within Cytoscape software, employing a confidence score threshold of ≥ 0.4. **B** Enrichment analysis of molecular functions, biological processes, and KEGG pathways was conducted for all genes within the PPI network using the ClueGO plugin in Cytoscape

intensities in the nucleus, cytoplasm, and membranous tissues samples, which were analyzed separately in this study (Table 2) (Fig. 4).

Although the median nuclear expression of DDIT4 was higher in cancer tissues than in non-malignant samples, it's not statistically significant. However, the median cytoplasmic and membranous expression of DDIT4 in tumors was lower than in non-malignant tissues. Besides, non-parametric Kruskal–Wallis and Mann–Whitney U tests were performed, which indicated a statistically significant difference between the median cytoplasmic and membranous expression of DDIT4 and the median expression of non-malignant tissues ($P = 0.007$ and $P = 0.002$, respectively). The Kruskal–Wallis and Mann–Whitney U tests demonstrated no significant difference between the median nuclear expression of DDIT4 protein in tumor samples and non-malignant samples ($P = 0.948$).

Table 1 Clinicopathological characteristic of patients with gastric cancer

Patients and tumor characteristics	Gastric cancer samples N (%)
Number of samples	213
Median age, years (Range)	63 (24–84)
≤ Median age	114 (53.8)
> Median age	98 (46.2)
Sex	
Male	158 (74.2)
Female	55 (25.8)
Histological subtypes	
Signet ring cell carcinoma	64 (30.0)
Intestinal type	149 (70.0)
Median tumor size (cm) (Range)	5.0 (1.0–15.0)
≤ Median	119 (60.4)
> Median	78 (39.6)
Histological grade	
Well differentiated	41 (20.8)
Moderate differentiated	54 (27.4)
Poor differentiated	102 (51.8)
Primary tumor (PT) stage	
pT1	50 (24.8)
pT2	65 (32.2)
pT3	78 (38.6)
pT4	9 (4.5)
Tumor extension	
Subserosa	95 (45.5)
Serosa	38 (18.2)
Others	76 (36.4)
Lamina propria	
Yes	209 (100)
No	0 (0.0)
Muscularis mucosa	
Yes	206 (98.6)
No	3 (1.4)
Submucosa	
Yes	192 (91.9)
No	17 (8.1)
Muscularis propria	
Yes	184 (88)
No	25 (12)
Subserosa	
Yes	147 (70.3)
No	62 (29.7)
Serosa	
Yes	54 (25.8)
No	155 (74.2)
Subserosa fat	
Yes	43 (70.5)
No	18 (29.5)
Margin	
Yes	14 (7.2)
No	181 (92.8)

Table 1 (continued)

Patients and tumor characteristics	Gastric cancer samples N (%)
Perineural invasion	
Present	92 (50.8)
Absent	89 (49.2)
Lymphovascular invasion	
Present	85 (42.3)
Absent	116 (57.7)
Surgery	
Radical	95 (44.6)
Partial	118 (55.4)
Recurrence	
Yes	38 (19.5)
No	157 (80.5)
Metastasis	
Yes	74 (37.9)
No	121 (62.1)

3.4 Expression of DDIT4 and its association with clinicopathological features of GC patients

Pearson's chi-square test and Spearman's correlation were used to examine association and correlation between expression of DDIT4 and clinicopathological features of patients with GC.

3.4.1 Nuclear DDIT4 expression

The results of Pearson's χ^2 test showed a significant association between increased nuclear expression of DDIT4 and advanced histological grade in term of intensity of staining and H-score (*P = 0.009 and P = 0.047*). We also found a statistically significant association between nuclear DDIT4 expression and histological subtypes in both intensity of staining and H-score (*P = 0.010 and P = 0.025* respectively) (Table 3).

3.4.2 Cytoplasmic DDIT4 expression

Subsequently, cytoplasmic analysis was performed, and the results of Pearson's χ^2 test showed a significant association between high cytoplasmic expression of DDIT4 and histological subtypes in terms of intensity and H-score (*P = 0.027, P = 0.001* respectively). The decrease in expression of DDIT4 was significantly associated with advanced histological grade in terms of H-scores (*P = 0.001*) (Table 4).

3.4.3 Membranous DDIT4 expression

The statistically significant association was observed between membranous low DDIT4 expression and increased histological grade in terms of intensity and H-score (*P = 0.035* and *P = 0.016*) as well as serosa invasion in terms of H-score (*P = 0.025*) (Table 5).

3.5 Information of clinical outcomes in patients with GC

In this study, 74 (37.9%) patients showed metastasis and 38 (19.5%) had recurrence. During the 114-month follow-up period, the mean time for DSS was 40 (SD = 26.65) and for PFS was 37 (26.82) months. Furthermore, the median of the follow-up was 38 (Q1, Q3 = 19, 57) and 36 (14, 56) months, and the range was 1–114 months for DSS or PFS, respectively. Cancer-related deaths occurred in 94 (48.2%) cases. The main features of the patients enrolled for survival analysis of GC were summarized in Table 6.

Table 2 Expression of DDIT4 in gastric cancer tissues and adjacent non-malignant tissue samples

Expression of DDIT4	Gastric cancer tissues N (%)	Adjacent non-malignant tissues N (%)	P-value
Nuclear expression			
Intensity of staining			
Negative (0)	72 (33.8)	12 (50.0)	0.210
Weak (+1)	5 (2.3)	0 (0.0)	
Moderate (+2)	47 (22.1)	4 (16.7)	
Strong (+3)	89 (41.8)	8 (33.3)	
Percentage of positive tumor cells			
< 25%	211 (99.1)	24 (100.0)	0.092
25–50%	2 (0.9)	0 (0.0)	
51–75%	0 (0.0)	0 (0.0)	
> 75%	0 (0.0)	0 (0.0)	
H-score cut off	15	5	
Low	167 (78.4)	12 (50.0)	0.150
High	46 (21.6)	12 (50.0)	
Cytoplasmic expression			
Intensity of staining			
Negative (0)	0 (0.0)	0 (0.0)	0.166
Weak (+1)	8 (3.8)	0 (0.0)	
Moderate (+2)	55 (25.8)	4 (16.7)	
Strong (+3)	150 (70.4)	20 (83.3)	
Percentage of positive tumor cells			
< 25%	0 (0.0)	0 (0.0)	***0.004***
25–50%	0 (0.0)	0 (0.0)	
51–75%	4 (1.9)	0 (0.0)	
> 75%	209 (98.1)	24 (100.0)	
H-score cut off	285	288	
Low	111 (52.1)	4 (16.7)	***0.007***
High	102 (47.9)	20 (83.3)	
Membranous expression			
Intensity of staining			
Negative (0)	130 (61.0)	8 (33.3)	***0.003***
Weak (+1)	1 (0.5)	0 (0.0)	
Moderate (+2)	34 (16.0)	4 (16.7)	
Strong (+3)	48 (22.5)	12 (50.0)	
Percentage of positive tumor cells			
< 25%	209 (98.1)	23 (95.8)	***0.003***
25–50%	4 (1.9)	1 (4.2)	
51–75%	0 (0.0)	0 (0.0)	
> 75%	0 (0.0)	0 (0.0)	
H-score cut off	11	17	
Low	130 (61.0)	12 (50.0)	***0.002***
High	83 (39.0)	12 (50.0)	

Values presented in bolditalic indicate statistical significance

3.6 Survival outcomes based on the expression of DDIT4 in the GC patients

Kaplan–Meier survival analysis indicated no significant differences between DSS or PFS and the patients with high and low nuclear and membranous expression of DDIT4 (Log-rank; DSS; *P = 0.921, P = 0.202, PFS; P = 0.973, P = 0.353*, respectively) (Fig. 5A, B, E, F). However, Kaplan–Meier curve results showed significant differences between the patients with high and low cytoplasmic expression rates of DDIT4 and DSS (Log-Rank test, *P = 0.038*), which suggests that high cytoplasmic expression of DDIT4 is associated with better DSS (Fig. 5C). Whereas there was no significant

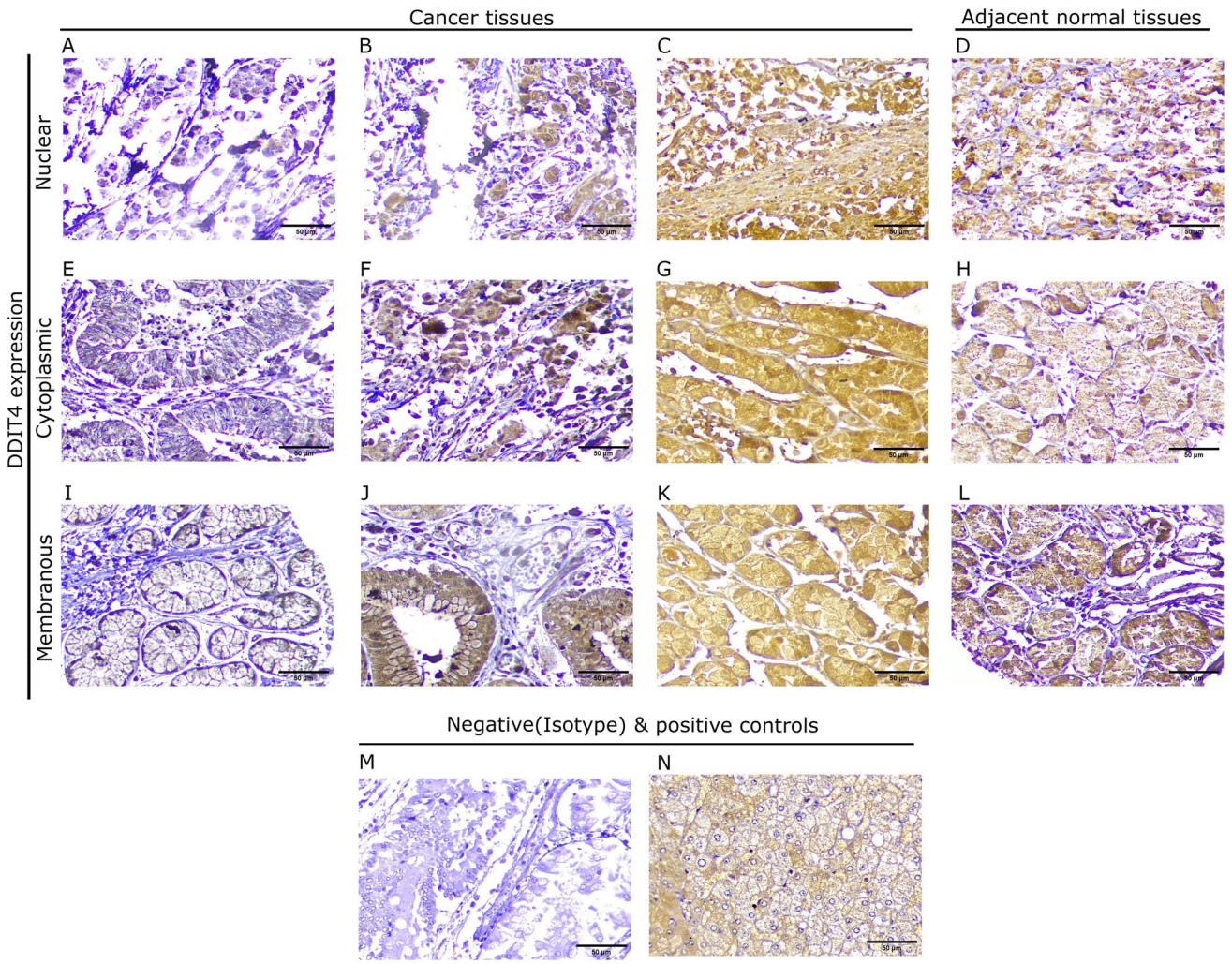

Fig. 4 Immunohistochemical analysis of DDIT4 expression in the nucleus, cytoplasm, and membrane of gastric cancer cells. DDIT4 expression in nucleus: **A** low, **B** moderate, **C** high, **D** Adjacent normal tissue. DDIT4 protein expression in cytoplasm: **E** low, **F** moderate, **G** high, **H** Adjacent normal tissue. DDIT4 protein expression in membrane: **I** low, **J** moderate, **K** high, **L** Adjacent normal tissue, **M** Isotype control. **N** Normal liver tissues as positive control for DDIT4 expression. Figures are shown with a magnification of 400×

difference between the cytoplasmic expression of DDIT4 and PFS (Log-Rank test, *P* = *0.197*) (Fig. 5D). Kaplan–Meier survival analysis showed no significant differences in DSS or PFS based on the intensity of staining or the percentage of positive cells across nuclear, cytoplasmic and membranous expressions of DDIT4. P value based on the intensity including: (Log-Rank test, DSS; P = 0.667, P = 0.794, P = 0.433, PFS; P = 0.289, P = 0.478, P = 0.638) (Fig. S1. A, B, C, D, E, F) and P value based on the percentage in order: (Log-Rank test, DSS; P = 0.224, P = 0.968, P = 0.365, PFS; P = 0.239, P = 0.875, P = 0.351) (Fig. S2. A, B, C, D, E, F).

Univariate and multivariate Cox regression analyses were performed to evaluate the clinical significance of potential prognostic factors for DSS and PFS. On univariate analyses, cytoplasmic expression of DDIT4 based on H-score (*P* = *0.046*), tumor size (*P* = *0.03*), serosa invasion (*P* = *0.021*), tumor recurrence (*P* = *0.004*), and distant metastasis (*P* < *0.001*) were detected as potential prognostic factors for DSS and PFS. As demonstrated in Table 7 distant metastasis was the only independent prognostic factor for DSS (P < 0.001) and PFS (HR: 0.155; 95% CI: 0.096–0.250; P < 0.001, respectively) (Table 7).

Table 3 The association between nuclear DDIT4 expression and clinicopathological characteristic of Gastric Cancer patients (P value; Pearson's χ^2 test)

Characteristics of tumor	Total samples N (%)	Intensity of staining N (%)				*P value*	H score (cut off = 15) N (%)		*P value*
		0 (Negative)	1 + (Weak)	2 + (Moderate)	3 + (Strong)		Low (≤ 15)	High (> 15)	
Median age, years (Range)	63 (24–84)								
≤ Median age	114 (53.8)	33 (45.8)	3 (60.0)	32 (68.1)	46 (52.3)	0.119	82 (49.1)	32 (71.1)	***0.009***
> Median age	98 (46.2)	39 (54.2)	2 (40.0)	15 (31.9)	42 (47.7)		85 (50.9)	13 (28.9)	
Sex									
Male	158 (74.2)	54 (75.0)	4 (80.0)	33 (70.2)	67 (75.3)	0.906	122 (73.1)	36 (78.3)	0.475
Female	55 (25.8)	18 (25.0)	1 (20.0)	14 (29.8)	22 (24.7)		45 (26.9)	10 (21.7)	
Histological subtypes									
Signet Ring Cell Carcinoma	64 (30.0)	15 (20.8)	4 (80.0)	19 (40.4)	26 (29.2)	***0.010***	44 (26.3)	20 (43.5)	***0.025***
Intestinal Type	149 (70.0)	57 (79.2)	1 (20.0)	28 (59.6)	63 (70.8)		123 (73.7)	26 (56.5)	
Median tumor size (cm) (Range)	5.0 (1.0–15.0)								
≤ Median	119 (60.4)	45 (68.2)	2 (40.0)	24 (57.1)	48 (57.1)	0.376	91 (59.9)	28 (62.2)	0.777
> Median	78 (39.6)	21 (31.8)	3 (60.0)	18 (42.9)	36 (42.9)		61 (40.1)	17 (37.8)	
Histological grade									
Well differentiated	41 (20.8)	24 (35.3)	0 (0.0)	5 (11.9)	12 (14.6)	***0.009***	35 (22.9)	6 (13.6)	***0.047***
Moderate differentiated	54 (27.4)	17 (25.0)	1 (20.0)	9 (21.4)	27 (32.9)		46 (30.1)	8 (18.2)	
Poor differentiated	102 (51.8)	27 (39.7)	4 (80.0)	28 (66.7)	43 (52.4)		72 (47.1)	30 (68.2)	
Primary tumor (PT) stage									
pT1	50 (24.8)	21 (31.8)	1 (20.0)	8 (17.8)	20 (23.3)	0.885	41 (26.1)	9 (20.0)	0.330
pT2	65 (32.2)	18 (27.3)	2 (40.0)	15 (33.3)	30 (34.9)		49 (31.2)	16 (35.6)	
pT3	78 (38.6)	25 (37.9)	2 (40.0)	19 (42.2)	32 (37.2)		62 (39.5)	16 (35.6)	
pT4	9 (4.5)	2 (3.0)	0 (0.0)	3 (6.7)	4 (4.7)		5 (3.2)	4 (8.9)	
Tumor extension									
Subserosa	95 (45.5)	25 (36.2)	3 (60.0)	24 (52.2)	43 (48.3)	0.289	71 (43.6)	24 (52.2)	***0.048***
Serosa	38 (18.2)	13 (18.8)	1 (20.0)	11 (23.9)	13 (14.6)		26 (16.0)	12 (26.1)	
Others	76 (36.4)	31 (44.9)	1 (20.0)	11 (23.9)	33 (37.1)		66 (40.5)	10 (21.7)	
Lamina propria									
Yes	209 (100)	69 (100.0)	5 (100.0)	46 (100.0)	89 (100.0)	–[a]	163 (100.0)	46 (100.0)	–[a]
No	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Muscularis mucosa									
Yes	206 (98.6)	69 (100.0)	5 (100.0)	46 (100.0)	86 (96.6)	***0.250***	162 (99.4)	44 (95.7)	0.060
No	3 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.4)		1 (0.6)	2 (4.3)	
Submucosa									
Yes	192 (91.9)	61 (88.4)	5 (100.0)	46 (100.0)	80 (89.9)	***0.107***	150 (92.0)	42 (91.3)	0.875
No	17 (8.1)	8 (11.6)	0 (0.0)	0 (0.0)	9 (10.1)		13 (8.0)	4 (8.7)	

Table 3 (continued)

Characteristics of tumor	Total samples N (%)	Intensity of staining N (%)				*P value*	H score (cut off = 15) N (%)		*P value*
		0 (Negative)	1 + (Weak)	2 + (Moderate)	3 + (Strong)		Low (≤ 15)	High (> 15)	
Muscolarispropia									
Yes	184 (88)	58 (84.1)	5 (100.0)	45 (97.8)	76 (85.4)	0.090	142 (87.1)	42 (91.3)	0.440
No	25 (12)	11 (15.9)	0 (0.0)	1 (2.2)	13 (14.6)		21 (12.9)	4 (8.7)	
Subserosa									
Yes	147 (70.3)	44 (63.8)	4 (80.0)	35 (76.1)	64 (71.9)	0.478	111 (68.1)	36 (78.3)	0.183
No	62 (29.7)	25 (36.2)	1 (20.0)	11 (23.9)	25 (28.1)		52 (31.9)	10 (21.7)	
Serosa									
Yes	54 (25.8)	19 (27.5)	1 (20.0)	11 (23.9)	23 (25.8)	0.963	41 (25.2)	13 (28.3)	0.671
No	155 (74.2)	50 (72.5)	4 (80.0)	35 (76.1)	66 (74.2)		122 (74.8)	33 (71.7)	
Subserosal fat									
Yes	43 (70.5)	10 (66.7)	0 (0.0)	10 (76.9)	23 (69.7)	0.829	31 (70.5)	12 (70.6)	0.992
No	18 (29.5)	5 (33.3)	0 (0.0)	3 (23.1)	10 (30.3)		13 (29.5)	5 (29.4)	
Margin									
Yes	14 (7.2)	2 (2.9)	1 (20.0)	2 (5.0)	9 (11.0)	0.163	10 (6.5)	4 (9.5)	0.506
No	181 (92.8)	66 (97.1)	4 (80.0)	38 (95.0)	73 (89.0)		143 (93.5)	38 (90.5)	
Perineural invasion									
Present	92 (50.8)	30 (51.7)	0 (0.0)	18 (43.9)	44 (56.4)	0.116	75 (52.8)	17 (43.6)	0.307
Absent	89 (49.2)	28 (48.3)	4 (100.0)	23 (56.1)	34 (43.6)		67 (47.2)	22 (56.4)	
Lymphovascular invasion									
Present	85 (42.3)	29 (44.6)	3 (60.0)	20 (45.5)	33 (37.9)	0.649	65 (41.7)	20 (44.4)	0.740
Absent	116 (57.7)	36 (55.4)	2 (40.0)	24 (54.5)	54 (62.1)		91 (58.3)	25 (55.6)	
Metastasis									
Yes	74 (37.9)	29 (42.6)	2 (40.0)	17 (40.5)	26 (32.5)	0.622	57 (37.5)	17 (39.5)	0.808
No	121 (62.1)	39 (57.4)	3 (60.0)	25 (59.5)	54 (67.5)		95 (62.5)	26 (60.5)	
Recurrence									
Yes	38 (19.5)	11 (16.2)	0 (0.0)	16 (38.1)	11 (13.8)	***0.006***	27 (17.8)	11 (25.6)	0.253
No	157 (80.5)	57 (83.8)	5 (100.0)	26 (61.9)	69 (86.3)		125 (82.2)	32 (74.4)	

Values presented in bolditalic indicate statistical significance

[a]No statistics are computed because the parameter is constant

Table 4 The association between cytoplasmic DDIT4 protein expression and clinicopathological characteristic of Gastric Cancer patients (P value; Pearson's $\chi 2$ test)

Characteristics of tumor	Total samples N (%)	Intensity of staining *N* (%)				*P value*	H score (cut off = 285) N (%)		*P value*
		0 (Negative)	1 + (Weak)	2 + (Moderate)	3 + (Strong)		Low (≤ 285)	High (> 285)	
Median age, years (Range)	63 (24–84)								
≤ Median age	114 (53.8)	0 (0.0)	4 (50.0)	33 (60.0)	77 (51.7)	0.558	62 (56.4)	52 (51.0)	0.432
> Median age	98 (46.2)	0 (0.0)	4 (50.0)	22 (40.0)	72 (48.3)		48 (43.6)	50 (49.0)	
Sex									
Male	158 (74.2)	0 (0.0)	7 (87.5)	39 (70.9)	112 (74.7)	0.587	83 (74.8)	75 (73.5)	0.836
Female	55 (25.8)	0 (0.0)	1 (12.5)	16 (29.1)	38 (25.3)		28 (25.2)	27 (26.5)	
Histological subtypes									
Signet ring cell carcinoma	64 (30.0)	0 (0.0)	4 (50.0)	23 (41.8)	37 (24.7)	***0.027***	44 (39.6)	20 (19.6)	***0.001***
Intestinal type	149 (70.0)	0 (0.0)	4 (50.0)	32 (58.2)	113 (75.3)		67 (60.4)	82 (80.4)	
Median tumor size (cm) (Range)	5.0 (1.0–15.0)								
≤ Median	119 (60.4)	0 (0.0)	2 (28.6)	32 (62.7)	85 (61.2)	0.211	58 (56.9)	61 (64.2)	0.292
> Median	78 (39.6)	0 (0.0)	5 (71.4)	19 (37.3)	54 (38.8)		44 (43.1)	34 (35.8)	
Histological grade									
Well differentiated	41 (20.8)	0 (0.0)	1 (12.5)	6 (12.2)	34 (24.3)	0.285	12 (11.5)	29 (31.2)	***0.001***
Moderate differentiated	54 (27.4)	0 (0.0)	1 (12.5)	15 (30.6)	38 (27.1)		27 (26.0)	27 (29.0)	
Poor differentiated	102 (51.8)	0 (0.0)	6 (75.0)	28 (57.1)	68 (48.6)		65 (62.5)	38 (40.9)	
Primary tumor (PT) stage									
pT1	50 (24.8)	0 (0.0)	1 (12.5)	11 (21.2)	38 (26.8)	0.852	22 (21.0)	28 (28.9)	0.535
pT2	65 (32.2)	0 (0.0)	4 (50.0)	16 (30.8)	45 (31.7)		35 (33.3)	30 (30.9)	
pT3	78 (38.6)	0 (0.0)	3 (37.5)	22 (42.3)	53 (37.3)		44 (41.9)	34 (35.1)	
pT4	9 (4.5)	0 (0.0)	0 (0.0)	3 (5.8)	6 (4.2)		4 (3.8)	5 (5.2)	
Tumor extension									
Subserosa	95 (45.5)	0 (0.0)	5 (62.5)	25 (48.1)	65 (43.6)	0.182	54 (50.5)	41 (40.2)	***0.035***
Serosa	38 (18.2)	0 (0.0)	2 (25.0)	13 (25.0)	23 (15.4)		23 (21.5)	15 (14.7)	
Others	76 (36.4)	0 (0.0)	1 (12.5)	14 (26.9)	61 (40.9)		30 (28.0)	46 (45.1)	
Lamina propria									
Yes	209 (100)	0.(0.0)	8 (100.0)	52 (100.0)	149 (100.0)	–[a]	107 (100.0)	102 (100.0)	–[a]
No	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Muscularis mucosa									
Yes	206 (98.6)	0 (0.0)	8 (100.0)	52 (100.0)	146 (98.0)	0.542	105 (98.1)	101 (99.0)	0.589
No	3 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.0)		2 (1.9)	1 (1.0)	
Submucosa									
Yes	192 (91.9)	0.(0.0)	8 (100.0)	50 (96.2)	134 (89.9)	0.255	100 (93.5)	92 (90.2)	0.389
No	17 (8.1)	0 (0.0)	0 (0.0)	2 (3.8)	15 (10.1)		7 (6.5)	10 (9.8)	

Table 4 (continued)

Characteristics of tumor	Total samples N (%)	Intensity of staining *N* (%)				*P value*	H score (cut off = 285) N (%)		*P value*
		0 (Negative)	1 + (Weak)	2 + (Moderate)	3 + (Strong)		Low (≤ 285)	High (> 285)	
Muscolarispropia									
Yes	184 (88)	0.(0.0)	8 (100.0)	49 (94.2)	127 (85.2)	0.129	97 (90.7)	87 (85.3)	0.233
No	25 (12)	0 (0.0)	0 (0.0)	3 (5.8)	22 (14.8)		10 (9.3)	15 (14.7)	
Subserosa									
Yes	147 (70.3)	0.(0.0)	7 (87.5)	38 (73.1)	102 (68.5)	0.456	80 (74.8)	67 (65.7)	0.151
No	62 (29.7)	0 (0.0)	1(12.5)	14 (26.9)	47 (31.5)		27 (25.2)	35 (34.3)	
Serosa									
Yes	54 (25.8)	0.(0.0)	2 (25.0)	13 (25.0)	39 (26.2)	0.985	27 (25.2)	27 (26.5)	0.838
No	155 (74.2)	0 (0.0)	6 (75.0)	39 (75.0)	110 (73.8)		80 (74.8)	75 (73.5)	
Subserosal fat									
Yes	43 (70.5)	0 (0.0)	0 (0.0)	11 (78.6)	32 (68.1)	0.450	25 (80.6)	18 (60.0)	0.077
No	18 (29.5)	0 (0.0)	0 (0.0)	3 (21.4)	15 (31.9)		6 (19.4)	12 (40.0)	
Margin									
Yes	14 (7.2)	0 (0.0)	1 (12.5)	2 (4.2)	11 (7.9)	0.575	7 (6.9)	7 (7.5)	0.858
No	181 (92.8)	0 (0.0)	7 (87.5)	46 (95.8)	128 (92.1)		95 (93.1)	86 (92.5)	
Perineural invasion									
Present	92 (50.8)	0 (0.0)	2 (28.6)	25 (53.2)	65 (51.2)	0.473	48 (51.1)	44 (50.6)	0.948
Absent	89 (49.2)	0 (0.0)	5 (71.4)	22 (46.8)	62 (48.8)		46 (48.9)	43 (49.4)	
Lymphovascular invasion									
Present	85 (42.3)	0 (0.0)	6 (75.0)	26 (53.1)	53 (36.8)	***0.022***	48 (46.2)	37 (38.1)	0.251
Absent	116 (57.7)	0 (0.0)	2 (25.0)	23 (46.9)	91(63.2)		56 (53.8)	60 (61.9)	
Metastasis									
Yes	74 (37.9)	0 (0.0)	3 (37.5)	21 (42.0)	50 (36.5)	0.790	43 (43.0)	31 (32.6)	0.136
No	121 (62.1)	0 (0.0)	5 (62.5)	29 (58.0)	87 (63.5)		57 (57.0)	64 (67.4)	
Recurrence									
Yes	38 (19.5)	0 (0.0)	0 (0.0)	14 (28.0)	24 (17.5)	0.101	22 (22.0)	16 (16.8)	0.363
No	157 (80.5)	0 (0.0)	8 (100.0)	36 (72.0)	113 (82.5)		78 (78.0)	79 (83.2)	

Values presented in bolditalic indicate statistical significance

[a]No statistics are computed because the parameter is constant

Table 5 The association between membranous DDIT4 protein expression and clinicopathological characteristic of Gastric Cancer patients (P value; Pearson's χ^2 test)

Characteristics of tumor	Total samples N (%)	Intensity of staining *N* (%)				*P value*	H score (cut off = 11) N (%)		*P value*
		0 (Negative)	1 + (Weak)	2 + (Moderate)	3 + (Strong)		Low (≤ 11)	High (> 11)	
Median age, years (Range)	63 (24–84)								
≤ Median age	114 (53.8)	72 (55.8)	0 (0.0)	18 (52.9)	24 (50.0)	0.645	72 (55.8)	42 (50.6)	0.458
> Median age	98 (46.2)	57 (44.2)	1 (100.0)	16 (47.1)	24 (50.0)		57 (44.2)	41 (49.4)	
Sex									
Male	158 (74.2)	102 (78.5)	1 (100.0)	24 (70.6)	31 (64.6)	0.248	102 (78.5)	56 (67.5)	0.074
Female	55 (25.8)	28 (21.5)	0 (0.0)	10 (29.4)	17 (35.4)		28 (21.5)	27 (32.5)	
Histological subtypes									
Signet ring cell carcinoma	64 (30.0)	45 (34.6)	0 (0.0)	9 (26.5)	10 (20.8)	0.276	45 (34.6)	19 (22.9)	0.069
Intestinal type	149 (70.0)	85 (65.4)	1 (100.0)	25 (73.5)	38 (79.20)		85 (65.4)	64 (77.1)	
Median tumor size (cm) (Range)	5.0 (1.0–15.0)								
≤ Median	119 (60.4)	72 (59.5)	0 (0.0)	21 (65.6)	26 (60.5)	0.587	72 (59.5)	47 (61.8)	0.744
> Median	78 (39.6)	49 (40.5)	1 (100.0)	11 (34.4)	17 (39.5)		49 (40.5)	29 (38.2)	
Histological grade									
Well differentiated	41 (20.8)	18 (14.6)	0 (0.0)	7 (24.1)	16 (36.4)	***0.035***	18 (14.6)	23 (31.1)	***0.016***
Moderate differentiated	54 (27.4)	34 (27.6)	1 (100.0)	10 (34.5)	9 (20.5)		34 (27.6)	20 (27.0)	
Poor differentiated	102 (51.8)	71 (57.7)	0 (0.0)	12 (41.4)	19 (43.2)		71 (57.7)	31 (41.9)	
Primary tumor (PT) stage									
pT1	50 (24.8)	29 (23.2)	0 (0.0)	7 (22.6)	14 (31.1)	0.471	29 (23.2)	21 (27.3)	0.183
pT2	65 (32.2)	35 (28.0)	1 (100.0)	12 (38.7)	17 (37.8)		35 (28.0)	30 (39.0)	
pT3	78 (38.6)	54 (43.2)	0 (0.0)	12 (38.7)	12 (26.7)		54 (43.2)	24 (31.2)	
pT4	9 (4.5)	7 (5.6)	0 (0.0)	0 (0.0)	2 (4.4)		7 (5.6)	2 (2.6)	
Tumor extension									
Subserosa	95 (45.5)	54 (42.2)	1 (100.0)	19 (59.4)	21 (43.8)	0.246	54 (42.2)	41 (50.6)	0.195
Serosa	38 (18.2)	28 (21.9)	0 (0.0)	5 (15.6)	5 (10.4)		28 (21.9)	10 (12.3)	
Others	76 (36.4)	46 (35.9)	0 (0.0)	8 (25.0)	22 (45.8)		46 (35.9)	30 (37.0)	
Lamina propria									
Yes	209 (100)	128 (100.0)	1 (100.0)	32 (100.0)	48 (100.0)	–[a]	128 (100.0)	81 (100.0)	–[a]
No	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Muscularis mucosa									
Yes	206 (98.6)	125 (97.7)	1 (100.0)	32 (100.0)	48 (100.0)	0.588	125 (97.7)	81 (100.0)	0.165
No	3 (1.4)	3 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)		3 (2.3)	0 (0.0)	
Submucosa									
Yes	192 (91.9)	116 (90.6)	1 (100.0)	31 (96.9)	44 (91.7)	0.699	116 (90.6)	76 (93.8)	0.409
No	17 (8.1)	12 (9.4)	0 (0.0)	1 (3.1)	4 (8.3)		12 (9.4)	5 (6.2)	

Table 5 (continued)

Characteristics of tumor	Total samples N (%)	Intensity of staining *N* (%)				*P value*	H score (cut off = 11) N (%)		*P value*
		0 (Negative)	1 + (Weak)	2 + (Moderate)	3 + (Strong)		Low (≤ 11)	High (> 11)	
Muscolarispropia									
Yes	184 (88)	112 (87.5)	1 (100.0)	31 (96.9)	40 (83.3)	0.314	112 (87.5)	72 (88.9)	0.763
No	25 (12)	16 (12.5)	0 (0.0)	1 (3.1)	8 (16.7)		16 (12.5)	9 (11.1)	
Subserosa									
Yes	147 (70.3)	93 (72.7)	1 (100.0)	24 (75.0)	29 (60.4)	0.341	93 (72.7)	54 (66.7)	0.356
No	62 (29.7)	35 (27.3)	0 (0.0)	8 (25.0)	19 (39.6)		35 (27.3)	27 (33.3)	
Serosa									
Yes	54 (25.8)	40 (31.3)	0 (0.0)	5 (15.6)	9 (18.8)	0.151	40 (31.3)	14 (17.3)	***0.025***
No	155 (74.2)	88 (68.8)	1 (100.0)	27 (84.4)	39 (81.3)		88 (68.8)	67 (82.7)	
Subserosal fat									
Yes	43 (70.5)	32 (69.6)	0 (0.0)	5 (83.3)	6 (66.7)	0.757	32 (69.6)	11 (73.3)	0.781
No	18 (29.5)	14 (30.4)	0 (0.0)	1 (16.7)	3 (33.3)		14 (30.4)	4 (26.7)	
Margin									
Yes	14 (7.2)	9 (7.5)	0 (0.0)	0 (0.0)	5 (10.9)	0.362	9 (7.5)	5 (6.7)	0.826
No	181 (92.8)	111 (92.5)	1 (100.0)	28 (100.0)	41 (89.1)		111 (92.5)	70 (93.3)	
Perineural invasion									
Present	92 (50.8)	60 (51.3)	0 (0.0)	15 (60.0)	17 (44.7)	0.485	60 (51.3)	32 (50.0)	0.869
Absent	89 (49.2)	57 (48.7)	1 (100.0)	10 (40.0)	21 (55.3)		57 (48.7)	32 (50.0)	
Lymphovascular invasion									
Present	85 (42.3)	56 (44.8)	0 (0.0)	13 (44.8)	16 (34.8)	0.533	56 (44.8)	29(38.2)	0.355
Absent	116 (57.7)	69 (55.2)	1 (100.0)	16 (55.2)	30 (65.2)		69 (55.2)	47 (61.8)	
Metastasis									
Yes	74 (37.9)	48 (40.0)	0 (0.0)	12 (42.9)	14 (30.4)	0.529	48 (40.0)	26 (34.7)	0.455
No	121 (62.1)	72 (60.0)	1 (100.0)	16 (57.1)	32 (69.6)		72 (60.0)	49 (65.3)	
Recurrence									
Yes	38 (19.5)	22 (18.3)	0 (0.0)	6 (21.4)	10 (21.7)	0.906	22 (18.3)	16 (21.3)	0.607
No	157 (80.5)	98 (81.7)	1 (100.0)	22 (78.6)	36 (78.3)		98 (81.7)	59 (78.7)	

Values presented in bolditalic indicate statistical significance

[a]No statistics are computed because the parameter is constant

Table 6 The main characteristics of patients enrolled for survival analysis according to total samples of gastric cancer

Features	Total sample of GC, N (%)
Number of patients (N)	195
Range of follow-up duration for DSS or PFS (months)	1–114, 1–114
Mean duration of follow-up time for DSS or PFS (months) (SD)	40 (26.65), 37 (26.82)
Median duration of follow-up time for DSS or PFS (months) (Q1, Q3)	38 (19, 57), 36 (14, 56)
Cancer-related death (N %)	94 (48.2)
Other causes of death (N %)	9 (4.6)
Distant metastasis during follow-up (N %)	74 (37.9)
Tumor recurrence during follow-up (N %)	38 (19.5)

4 Discussion

GC remains an important cancer worldwide and imposes a substantial health-related and economic burden on patients and society [36]. Despite major improvements in patient survival over the past few decades, the diagnosis and prognosis of patients with GC are still unsatisfactory [37]. GC patients are typically diagnosed at advanced stages and have a high recurrence rate [2].Thus, the identification of novel molecules is required for gastric tumor diagnosis, clinical stage determination, treatment response evaluation, and recurrence screening after treatment [38].

Based on the STRING-PPI network, DDIT4 correlates with proteins that have important roles in cancer, such as TP53, TSC1/2, and SFN (14-3-3). TSC1/2 are known as tumor suppressors that control cell growth and proliferation. Under conditions of cell stress, the expression of DDIT4 increases, leading to the formation of an active TSC1/2 complex, which inhibits mTORC1 and cell proliferation [18, 39, 40]. In addition, SFN (14-3-3) is highly expressed in several malignancies and is associated with a poor prognosis [41–43].

Furthermore, according to a literature review and data mining by KEGG, DDIT4 is involved in the PI3K-Akt signaling pathway in cancer [40]. Recent studies have highlighted the importance of DDIT4 in a variety of human cancer types [23, 44, 45]. mTORC1 has been identified as the most important downstream target for this protein [40]. mTORC1 is a multiprotein complex that regulates protein translation, cell growth, and metabolism [18]. Until now, inconsistent roles for DDIT4 in cell death and carcinogenesis have been reported. In vitro and in vivo studies show that the upregulation of DDIT4 is associated with increased cell proliferation, invasion, migration, and decrease in apoptosis in cancer cells [14, 24, 46]. High levels of DDIT4 can protect cancer cells against hypoxia-induced cell death [25, 47, 48]. In addition, some studies have shown an association between DDIT4 expression and the increased production of the anti-apoptotic protein BCL2, which results in alterations in p53 phosphorylation that reduce apoptosis [15, 48]. Also, other studies revealed that silencing DDIT4 makes tumor cells more sensitive to chemotherapy [14, 26, 28, 29]. The antitumor role of DDIT4 has also been shown in several studies. In response to DNA damage, the p53 protein upregulates DDIT4 expression, which inhibits mTORC1 and ultimately promotes cell death [9, 14, 45].

Bioinformatics analysis showed that there is a significant difference in the mRNA expression of DDIT4 between GC and normal tissues. In this current study, cytoplasmic and membranous expression of DDIT4 were statistically lower in GC tissue than in non-malignant tissue, while increased nuclear expression of DDIT4 compared to non-malignant tissues was observed in GC, which was consistent with the findings of Chang et al. and Fattahi et al., who observed increased nuclear expression of DDIT4 in OC tissues and CRC, respectively, compared to normal tissues [5, 23]. In normal cells, DDIT4 is mainly expressed in the cytoplasm [14]. Given that mTOR regulates cell proliferation and that DDIT4 inhibits mTOR [11]. Based on our results, decreased cytoplasmic expression of DDIT4 in cancer cells may results in mTORC1 activation and promote tumor growth.

It has been proposed that DDIT4 may have different functions in subcellular localization [23, 45]. Our results showed that DDIT4 is expressed in the nucleus of tumor cells. Nevertheless, there is limited reports on nuclear expression of DDIT4 and no report has been published about the function of DDIT4 in the nucleus. It has been reported that, in diffuse large B-cell lymphoma cells, DDIT4 induction by the noncanonical NF-κB pathway, enhanced DNA repair, suppressed centrosome amplification, and maintained genome integrity [46]. DNA damage is a well-known factor in the progression of cancer, and DNA repair is essential for cancer cell survival [47]. Therefore, it can be supposed that the high nuclear expression of DDIT4 is a result of its mediation in DNA repair in the nucleus of cancer cells. There are several molecules involved in DNA repair that, in cancerous conditions, can also change their localization

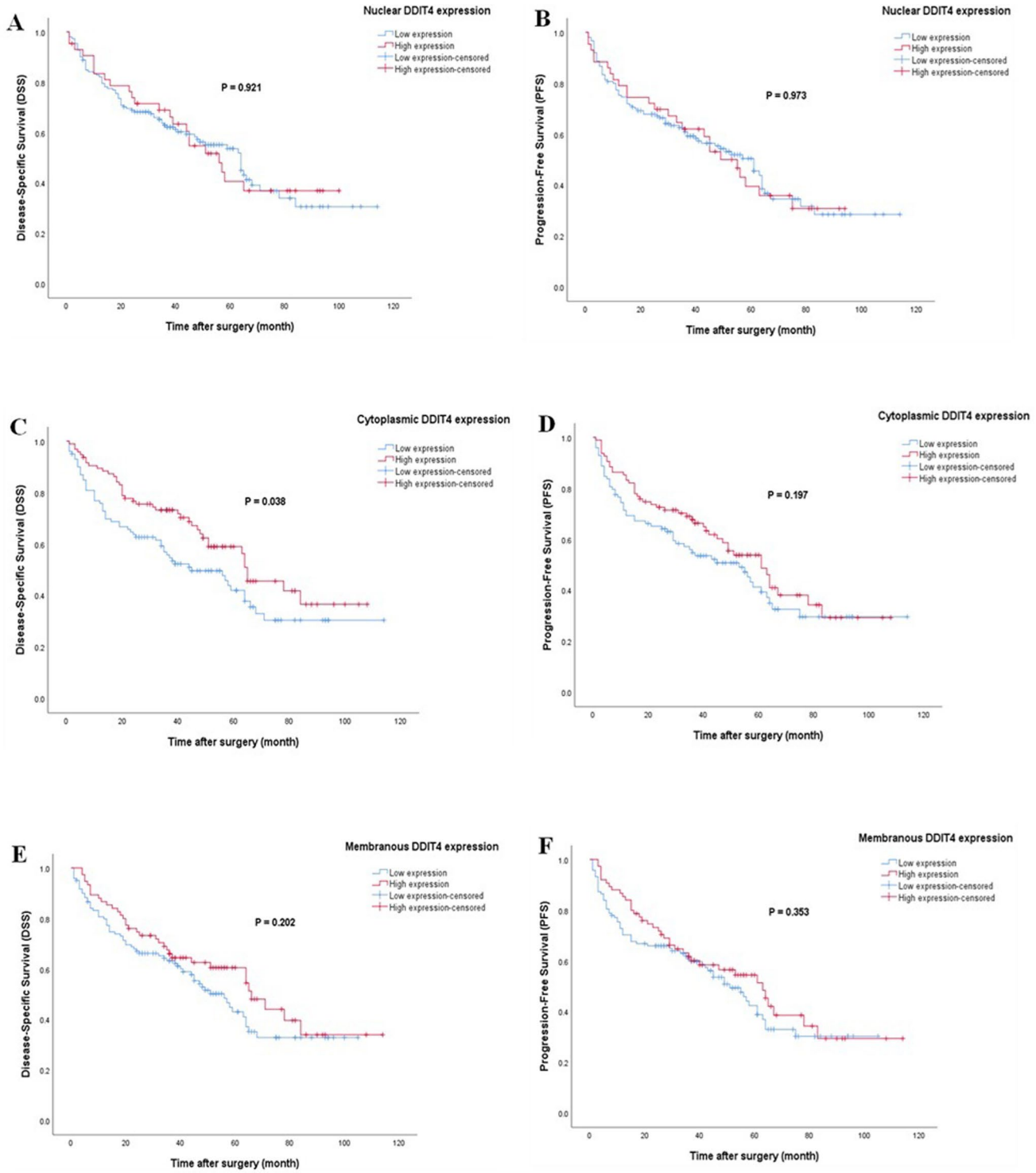

Fig. 5 Kaplan–Meier survival analysis for disease-specific survival (DSS) and progression-free survival (PFS) of Gastric Cancer (GC) patients based on high and low nuclear, membranous, and cytoplasmic expression levels of DDIT4. **A**, **B** No statistically significant differences were observed in DSS (Log-Rank test; *P = 0.921*) and PFS (Log-Rank test; *P = 0.973*) between the patients with high and low nuclear expression of DDIT4 in tumor cells. **C** Statistically significant differences were noted in DSS (Log-Rank test; *P = 0.038*) between patients with high and low cytoplasmic expression of DDIT4 in tumor cells; **D** No statistically significant differences were found in PFS (Log-Rank test; *P = 0.197*) between the patients with high and low cytoplasmic expression of DDIT4 in tumor cells. **E, F** No statistically significant differences were observed in DSS (Log-Rank test; *P = 0.202*) and PFS (Log-Rank test; *P = 0.353*) between the patients with high and low membranous expression of DDIT4 in tumor cells

Table 7 Univariate and multivariate cox regression analyses of potential prognostic factors for disease-Specific survival (DSS), and progression-Free Survival (PFS) in patients with gastric cancer

Covariate	Disease-Specific survival (DSS)				Progression-Free Survival (PFS)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*
Age (years)	0.914 (0.610–1.368)	0.661	–	–	0.904 (0.610–1.340)	0.615	–	–
Tumor size (cm)	1.583 (1.046–2.395)	***0.030***	1.147 (0.750–1.756)	0.527	1.617 (1.078–2.426)	***0.020***	1.254 (0.827–1.901)	0.287
Histological grade	0.680 (0.381–1.216)	0.401	–	–	0.733 (0.423–1.270)	0.540	–	–
Moderate versus well	0.991 (0.627–1.565)	0.194			0.940 (0.598–1.477)	0.268		
Poor versus well		0.969				0.788		
TNM stage	0.813 (0.276–2.395)	0.204	–	–	0.734 (0.275–1.957)	0.308	–	–
I versus II	0.998 (0.351–2.837)	0.708			0.929 (0.362–2.382)	0.536		
I versus III	1.414 (0.505–3.956)	0.997			1.212 (0.478–3.872)	0.878		
I versus IV		0.510				0.686		
Muscularis mucosa	0.471 (0.066–3.382)	0.454	–	–	0.426 (0.059–3.059)	0.396	–	–
Submucosa	0.683 (0.299–1.561)	0.366	–	–	0.724 (0.336–1.561)	0.410	–	–
Muscularis propria	0.645 (0.325–1.282)	0.211	–	–	0.775 (0.414–1.450)	0.425	–	–
Subserosa	0.809 (0.518–1.262)	0.350	–	–	0.826 (0.537–1.272)	0.386	–	–
Serosa	0.594 (0.381–0.925)	***0.021***	0.880 (0.540–1.434)	0.607	0.539 (0.352–0.826)	***0.005***	0.812 (0.509–1.296)	0.383
Sub serosal fat	0.748 (0.318–1.762)	0.507	–	–	0.776 (0.343–1.756)	0.543	–	–
Macroscopic tumor extension	1.018 (0.819–1.266)	0.870	–	–	1.066 (0.863–1.318)	0.552	–	–
Margin	0.821 (0.379–1.780)	0.617	–	–	0.925 (0.427–2.001)	0.842	–	–
Perineural invasion	0.949 (0.624–1.442)	0.805	–	–	0.873 (0.580–1.314)	0.514	–	–
Lymphovascular invasion	0.903 (0.594–1.372)	0.632	–	–	0.872 (0.581–1.309)	0.509	–	–
Tumor recurrence	0.532 (0.344–0.822)	***0.004***	1.271 (0.756–2.140)	0.366	0.493 (0.316–0.768)	***0.002***	1.021 (0.618–1.684)	0.936
Distant metastasis	0.133 (0.085–0.208)	**<0.001**	0.124 (0.075–0.206)	***<0.001***	0.161 (0.106–0.245)	***<0.001***	0.155 (0.096–0.250)	***<0.001***
Nuclear DDIT4 expression (intensity)	0.947 (0.591–1.518)	0.674	–	–	0.755 (0.477–1.197)	0.319	–	–
0 versus 1	0.758 (0.183–3.147)	0.821			0.304 (0.042–2.209)	0.232		
0 versus 2	1.266 (0.770–2.081)	0.703			1.100 (0.679–1.780)	0.239		
0 versus 3		0.352				0.699		
Nuclear DDIT4 expression (percentage of positive cells) Group 2 (25%-49%) versus group 1 (< 25%)	0.432 (0.106–1.759)	0.241	–	–	2.256 (0.554–9.182)	0.256	–	–
Nuclear DDIT4 expression High versus low	0.828 (0.508–1.348)	0.448	–	–	1.199 (0.811–1.773)	0.362	–	–
Cytoplasmic DDIT4 expression (intensity)	0.760 (0.239–2.417)	0.797	–	–	0.445 (0.109–1.817)	0.498	–	–
1 versus 2	1.102 (0.708–1.715)	0.641			1.056 (0.685–1.627)	0.260		
1 versus 3		0.668				0.805		
Cytoplasmic DDIT4 expression (percentage of positive cells) Group 4 (> 75%) versus group 3 (50%-75%)	0.971 (0.239–3.948)	0.968	–	–	1.118 (0.275–4.542)	0.876	–	–

Table 7 (continued)

Covariate	Disease-Specific survival (DSS)				Progression-Free Survival (PFS)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*
Cytoplasmic DDIT4 expression High versus low	0.658 (0.436–0.993)	***0.046***	0.748 (0.474–1.181)	0.212	0.777 (0.527–1.145)	0.202	–	–
Membranous DDIT4 expression (intensity) 0 versus 1 0 versus 2 0 versus 3	1.427 (0.854–2.384) 1.786 (0.924–3.457) 1.292 (0.663–2.516)	0.604 0.175 0.969 0.451	–	–	1.209 (0.750–1.947) 1.104 (0.678–1.565) 1.064 (0.561–2.017)	0.875 0.436 0.966 0.850	–	–
Membranous DDIT4 expression (percentage of positive cells) Group 2 (25%-49%) versus group 1 (< 25%)	2.402 (0.335–17.239)	0.384	–	–	0.407 (0.057–2.920)	0.371	–	–
Membranous DDIT4 expression High versus low	0.775 (0.501–1.197)	0.251	–	–	0.821 (0.547–1.231)	0.340	–	–

Values presented in bolditalic indicate statistical significance

within the cell; for example, in response to DNA damage in cancer, the RAD51 protein localizes to the nucleus to participate in repair reactions [48]. Furthermore, in cancer cells, following cellular stress, cytoplasmic p53 relocate to the nucleus [49]. In the present study, a significant association was observed between decreased cytoplasmic DDIT4 expression and increased nuclear expression with a higher grade that is associated with more undifferentiated cells. The association between the increased expression of DDIT4 and decreased tumor cell differentiation has been previously reported by Chen and Fattahi [5, 50]. Histological grade is generally considered one of the most significant prognostic factors in cancer [51], and a higher grade is associated with decreased tumor differentiation and worse outcomes [52]. A previous study conducted by Kuo-Hao Ho et al. in 2020 on glioblastoma revealed that DDIT4 participates in GLUT3-mediated glucose metabolism. Overexpression of DDIT4 enhanced tumor sphere formation size of glioma cells. These findings showed that increased DDIT4 expression enhances the stemness properties mediated by GLUT3 through glycolytic metabolism [53]. Therefore, it can be concluded that the nuclear expression of DDIT4 leads to an increase in stemness characteristics, which is accompanied by a decrease in differentiation and an increase in tumor grade.

In relation to survival and its association with DDIT4 expression, data retrieved from TCGA for gastric adenocarcinoma revealed no difference in survival between groups with low and high DDIT4 expression (P-value in the log rank test of 0.999) [6]. In another study, univariate survival analysis found that DDIT4 was not associated with GC patients survival in a statistically meaningful manner [14]. Survival analysis showed a significant association between the patients with high and low cytoplasmic expression rates of DDIT4 and DSS (Log-Rank test, $P = 0.038$), which revealed that low cytoplasmic expression of DDIT4 is associated with worse DSS in GC patients. In-silico analysis, using pooled datasets from KM Plotter and meta-analysis of individual datasets from SurvExpress showed that high DDIT4 expression was associated with an improved prognosis in GC. In contrast, high DDIT4 expression was significantly associated with a poorer prognosis for several cancers, such as breast, colon, skin, and lung cancers [6]. In this regard, our results show that a decrease in cytoplasmic expression is associated with a worse prognosis.

However, the limitations of this study are small sample size and brief duration of follow-up to confirm the prognostic role of this marker, it is crucial to evaluate the expression of DDIT4 in a larger sample size and extend the duration of follow-up.

5 Conclusion

In this study, it was shown that low cytoplasmic expression and increased nuclear expression of DDIT4 in patients with GC are associated with disease progression as well as a poor prognosis.

In conclusion, the findings of this study highlighted low cytoplasmic expression of DDIT4 and nuclear overexpression of DDIT4 in GC tumor cells, which may indicate more aggressive tumor behavior and more advanced disease in patients with GC. Considering that the level of cytoplasmic expression has been related to DSS, this marker can be considered a prognostic marker in GC patients.

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Data availability Data is provided within the manuscript or supplementary information files.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The protocol was approved by Iran University of Medical Sciences Human Research Ethics Committee in accordance with the Ref No: IR.IUMS.REC.1402.181. At the time of sample collection, informed consent was obtained from all individual participants.

Competing interests The authors declare no competing interests.

References

1. Chhikara BS, Parang K. Global Cancer Statistics 2022: the trends projection analysis. Chem Biol Lett. 2023;10(1):451–451.
2. Wagner A, et al. Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev. 2017; (8): CS004604.
3. Sun W, Yan L. Gastric cancer: current and evolving treatment landscape. Chin J Cancer. 2016;35(1):1–5.
4. Zhou C, et al. Prognostic biomarkers for gastric cancer: an umbrella review of the evidence. Front Oncol. 2019;9:1321.
5. Fattahi F, et al. High expression of DNA damage-inducible transcript 4 (DDIT4) is associated with advanced pathological features in the patients with colorectal cancer. Sci Rep. 2021;11(1):1–17.
6. Pinto JA, et al. In silico evaluation of DNA Damage Inducible Transcript 4 gene (DDIT4) as prognostic biomarker in several malignancies. Sci Rep. 2017;7(1):1526.
7. Vega-Rubin-de-Celis S, et al. Structural analysis and functional implications of the negative mTORC1 regulator REDD1. Biochemistry. 2010;49(11):2491–501.
8. Shoshani T, et al. Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis. Mol Cell Biol. 2002;22(7):2283–93.
9. Ellisen LW, et al. REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. Mol Cell. 2002;10(5):995–1005.
10. Protiva P, et al. Growth inhibition of colon cancer cells by polyisoprenylated benzophenones is associated with induction of the endoplasmic reticulum response. Int J Cancer. 2008;123(3):687–94.
11. Sofer A, et al. Regulation of mTOR and cell growth in response to energy stress by REDD1. Mol Cell Biol. 2005;25(14):5834–45.
12. Wang Z, et al. Dexamethasone-induced gene 2 (dig2) is a novel pro-survival stress gene induced rapidly by diverse apoptotic signals. J Biol Chem. 2003;278(29):27053–8.
13. Inoki K, Corradetti MN, Guan K-L. Dysregulation of the TSC-mTOR pathway in human disease. Nat Genet. 2005;37(1):19–24.
14. Du F, et al. DDIT4 promotes gastric cancer proliferation and tumorigenesis through the p53 and MAPK pathways. Cancer Commun. 2018;38(1):1–14.
15. Chang B, et al. REDD1 is required for RAS-mediated transformation of human ovarian epithelial cells. Cell Cycle. 2009;8(5):780–6.
16. Lipina C, Hundal HS. Is REDD1 a metabolic eminence grise? Trends Endocrinol Metab. 2016;27(12):868–80.
17. Dennis MD, et al. REDD1 enhances protein phosphatase 2A–mediated dephosphorylation of Akt to repress mTORC1 signaling. Sci Signaling. 2014;7(335):ra68–ra68.
18. DeYoung MP, et al. Hypoxia regulates TSC1/2–mTOR signaling and tumor suppression through REDD1-mediated 14–3–3 shuttling. Genes Dev. 2008;22(2):239–51.
19. Gordon BS, et al. Emerging role for regulated in development and DNA damage 1 (REDD1) in the regulation of skeletal muscle metabolism. Am J Physiol-Endocrinol Metab. 2016;311(1):E157–74.
20. Horak P, et al. Negative feedback control of HIF-1 through REDD1-regulated ROS suppresses tumorigenesis. Proc Natl Acad Sci. 2010;107(10):4675–80.
21. Wang Y, et al. Baicalein upregulates DDIT4 expression which mediates mTOR inhibition and growth inhibition in cancer cells. Cancer Lett. 2015;358(2):170–9.
22. Kucejova B, et al. Interplay between pVHL and mTORC1 Pathways in clear-cell renal cell carcinoma deregulation of mTORC1 in ccRCC. Mol Cancer Res. 2011;9(9):1255–65.
23. Jin H-O, et al. Redd1 inhibits the invasiveness of non-small cell lung cancer cells. Biochem Biophys Res Commun. 2011;407(3):507–11.
24. Schwarzer R, et al. REDD1 integrates hypoxia-mediated survival signaling downstream of phosphatidylinositol 3-kinase. Oncogene. 2005;24(7):1138–49.
25. Chang B, et al. Overexpression of the recently identified oncogene REDD1 correlates with tumor progression and is an independent unfavorable prognostic factor for ovarian carcinoma. Diagn Pathol. 2018;13:1–12.
26. Zeng Q, et al. Inhibition of REDD1 sensitizes bladder urothelial carcinoma to paclitaxel by inhibiting autophagyInhibition of REDD1 sensitizes BUC to paclitaxel. Clin Cancer Res. 2018;24(2):445–59.
27. Jia W, et al. REDD1 and p-AKT over-expression may predict poor prognosis in ovarian cancer. Int J Clin Exp Pathol. 2014;7(9):5940.
28. Foltyn M, et al. The physiological mTOR complex 1 inhibitor DDIT4 mediates therapy resistance in glioblastoma. Br J Cancer. 2019;120(5):481–7.
29. Jin HO, et al. Induction of HSP27 and HSP70 by constitutive overexpression of Redd1 confers resistance of lung cancer cells to ionizing radiation. Oncol Rep. 2019;41(5):3119–26.
30. Chandrashekar DS, et al. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia. 2017;19(8):649–58.
31. Doncheva NT, et al. Cytoscape StringApp: network analysis and visualization of proteomics data. J Proteome Res. 2018;18(2):623–32.
32. Shannon P, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.

33. Safaei S, et al. Overexpression of cytoplasmic dynamin 2 is associated with worse outcomes in patients with clear cell renal cell carcinoma. Cancer Biomarkers. 2022(Preprint): p. 1–19.
34. McCarty KS Jr, et al. Use of a monoclonal anti-estrogen receptor antibody in the immunohistochemical evaluation of human tumors. Cancer Res. 1986;46(8_Supplement):4244s–8s.
35. Dziadziuszko R, et al. Correlation between MET gene copy number by silver in situ hybridization and protein expression by immunohistochemistry in non-small cell lung cancer. J Thorac Oncol. 2012;7(2):340–7.
36. Bray F, et al. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin. 2018;68(6):394–424.
37. Choi S, et al. Gastric cancer: mechanisms, biomarkers, and therapeutic approaches. Biomedicines. 2022;10(3):543.
38. Shimada H, et al. Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. Gastric Cancer. 2014;17:26–33.
39. Zhu Q-Y, et al. The role of TSC2 in breast cancer: a literature review. Front Oncol. 2023;13:1188371.
40. Mehta MS, et al. Polymorphic variants in TSC1 and TSC2 and their association with breast cancer phenotypes. Breast Cancer Res Treat. 2011;125:861–8.
41. Neal CL, et al. 14-3-3ζ overexpression defines high risk for breast cancer recurrence and promotes cancer cell survival. Can Res. 2009;69(8):3425–32.
42. Fan X, et al. 14-3-3 proteins are on the crossroads of cancer, aging, and age-related neurodegenerative disease. Int J Mol Sci. 2019;20(14):3518.
43. Neal CL, Yu D. 14-3-3ζ as a prognostic marker and therapeutic target for cancer. Expert Opin Ther Targets. 2010;14(12):1343–54.
44. Barakat DJ, et al. C/EBPβ regulates sensitivity to bortezomib in prostate cancer cells by inducing REDD1 and autophagosome–lysosome fusion. Cancer Lett. 2016;375(1):152–61.
45. Hwang-Verslues W, et al. miR-495 is upregulated by E12/E47 in breast cancer stem cells, and promotes oncogenesis and hypoxia resistance via downregulation of E-cadherin and REDD1. Oncogene. 2011;30(21):2463–74.
46. Smith ER, Xu X-X. REDD1, a new Ras oncogenic effector. Taylor & Francis; 2009.
47. Basu AK. DNA damage, mutagenesis and cancer. Int J Mol Sci. 2018;19(4):970.
48. Tarsounas M, Davies AA, West SC. RAD51 localization and activation following DNA damage. Philos Trans Royal Soc Lond Ser B Biol Sci. 2004;359(1441):87–93.
49. Sembritzki O, et al. Cytoplasmic localization of wild-type p53 in glioblastomas correlates with expression of vimentin and glial fibrillary acidic protein. Neuro Oncol. 2002;4(3):171–8.
50. Chen KS, et al. EGF receptor and mTORC1 are novel therapeutic targets in nonseminomatous germ cell tumors. Mol Cancer Ther. 2018;17(5):1079–89.
51. Matsuo K, et al. Association of tumor differentiation grade and survival of women with squamous cell carcinoma of the uterine cervix. J Gynecologic Oncol. 2018; 29(6).
52. Dicken BJ, et al. Gastric adenocarcinoma: review and considerations for future directions. Ann Surg. 2005;241(1):27.
53. Ho K-H, et al. A key role of DNA damage-inducible transcript 4 (DDIT4) connects autophagy and GLUT3-mediated stemness to desensitize temozolomide efficacy in glioblastomas. Neurotherapeutics. 2020;17:1212–27.