Lack of Relationship Between PROX1 Expression and Clinicopathological Parameters and Prognosis in Gastric Cancer Patients: A Meta-analysis and TCGA Analysis

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Research Article

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Abstract

Background:

The relationship between PROX1 expression and clinicopathological characteristics and prognosis in patients with gastric cancer (GC) is hotly contested and continues to be so. The aim of this study is to determine the clinicopathological and prognostic significance of PROX1 expression in patients with GC.

Methods:

PROX1 expression in GC patients was evaluated clinicopathologically and in terms of overall survival (OS) using a systematic literature search and meta-analysis. Additionally, the Cancer Genome Atlas (TCGA) and The Genotype-Tissue Expression (GTEx) datasets were utilized to examine the relationship between PROX1 expression and clinicopathological significance and overall survival (OS) in GC patients.

Results:

A total of 8 studies pooling 1289 GC patients were included in the assessment. PROX1 expression, in GC patients, was shown to be unrelated to gender (odds ratio (OR) : 1.234, 95%CI: 0.958-1.590, P = 0.104), depth of tumor invasion (OR: 0.742, 95%CI:0.428-1.287, P = 0.289), lymph node metastasis (OR: 2.161, 95%CI: 0.808-5.779, P = 0.125), TNM stage (OR: 1.324, 95%CI: 0.572-3.066, P = 0.513), tumor size (OR: 0.889, 95%CI: 0.502-1.576, P = 0.687), metastasis (OR: 1.096, 95%CI: 0.470-2.555, P= 0.763), 1-year OS (OR: 0.908, 95%CI: 0.631-1.306, P = 0.602), 3-years OS (OR: 1.234, 95%CI: 0.482-3.160, P = 0.661) and 5-years OS (OR: 0.853, 95%CI: 0.266-2.736, P = 0.790). Patients with high PROX1 expression had a worse OS than those with low PROX1 expression, according to TCGA analyses, however the difference was not statistically significant (p=0.119).

Conclusion:

The expression of PROX1 was shown to be unrelated to gender, TNM stage, depth of invasion, tumor size, stage, tumor cell metastasis, or lymph node metastasis. The expression of PROX1 was also unrelated to OS and it failed to be a meaningful biomarker to prevent and diagnose GC.

Introduction

As a class of primary cancer worldwide, gastric cancer (GC) is ranked fifth for incidence and fourth for mortality, which rates are twice over in men than in women (1). GC imposed a significant burden on personal health as well as societies and economies. Although the application of physical examination and gastroscopy has declined the incidence of GC over the past decade in most populations, the majority of GC patients are at a progressive stage when they have been diagnosed (2–3). No diagnostic biomarkers can be used for secondary prevention (4). This may contribute to high mortality. So a prognostic biomarker that enables earlier diagnosis of GC is urgently needed.

Prospero-related homeobox 1 (PROX1), a vertebrate homologue of Drosophila prospero, is a homeobox gene that encodes a transcription factor and a divergent homeodomain protein (5). PROX1 plays a pivotal role in various developmental processes of many organisms, and its signaling controls cell proliferation, differentiation, and apoptosis (6). PROX1 expression has been linked to carcinogenesis and prognosis in recent years, according to a rising number of studies. The PROX1 transcription factor, reported by Petrova TV *et al*. (7), can promote colon cancer development by facilitating the shift from a benign to a highly dysplastic phenotype. Prox1 mediates the antiproliferative impact of IFN- in esophageal cancer cells, reported by Akagami M *et al*., and Prox1 might be a viable target for new esophageal cancer treatment methods (8). Recently, studies suggest that there is a relationship between PROX1 and GC in clinicopathological and prognostic terms. Ueta K *et al*. (9) reported that the overexpression of PROX1 correlates positively with advanced TNM, lymphatic metastasis and poor prognosis. However, Alli Laitinen et al. (10) reported that the expression of PROX1 was irrelevant to them. These conclusions between PROX1 and GC are widely disputed and remain controversial. Thus, a comprehensive meta-analysis between the expression of PROX1 and GC in clinicopathological and prognostic is warranted.

Methods

Search strategy

We selected studies published until August 10, 2021, by searching Pubmed, Embase, Cochrane Library, Web of science, ClinicalTrials.gov and Chinese databases (WanFang, CNKI, WeiPu and CBM). No specific restrictions were applied, such as date, age, gender, or language. The search method consisted of two main components, which were linked together via AND: (I) Stomach Neoplasms (eg, Stomach Neoplasm, Gastric Cancer, Stomach Cancer), (II) prospero-related homeobox 1 (eg, PROX1, prox-1, Prospero homeobox 1)". To search, controlled vocabulary (i.e., Medical Subject Headings [MeSH] terms) and keywords associated with either of two main components were completely utilized. The search was originally developed for PubMed and then applied to the remaining 8 databases. We also performed a manual search using the reference list of major articles.

Study selection

The following studies were identified for inclusion: (I) The full text of the studies is available; (II) in GC patients, the relationship between PROX1 expression, clinicopathological characteristics, and prognosis was investigated.

The following studies were identified for exclusion: (I) animal testing; (II) cell experiments; (III) repeated studies using the same data or patients; (IV) adjuvant chemoradiation was administered to the patients; and (V) the research content is unrelated to the topic. The Newcastle-Ottawa (NOS) scale was used to evaluate manuscript quality. The NOS ratings ranged from 0 to 9, with a score of 6 indicating excellent quality. NOS ratings greater than 6 are regarded as excellent quality scores and will be added to our meta-analysis.

Data extraction

Two authors (Zirui Jia and Yuhang Wang) extract information about the study's characteristics such as year of publication, first author, location, study period and so on. Two reviewers (Zirui Jia and Yuhang Wang) independently extracted results from each eligible studies: gender, TNM stage, depth of invasion, tumor size, stage, tumor cell metastasis and lymph node metastasis and prognostic overall survival (OS) in 1, 3 and 5 years. The two reviewers resolved all differences through discussion.

The Cancer Genome Atlas Analysis

The Cancer Genome Atlas (TCGA) database was used to obtain tumor RNA-seq and clinicopathological parameter information for 375 GC patients, as well as 32 pairs of mRNA expression data in normal tissue samples. Other data from 359 normal tissue samples from the stomach were obtained from The Genotype-Tissue Expression (GTEx) (https://gtexportal.org/home/datasets). Like the TCGA database, complete information on normal tissue was offered by GTEx. Statistical analyses of PROX1 expression in GC and normal tissues were performed using R software. Survival analysis uses the Kaplan-Meyer method and a logarithmic test. Statistical significance is defined as a p-value of less than 0.05.

Statistical analysis

Stata 14.0 was used to conduct all of the analyses. The heterogeneity of the included studies was assessed using the q test and the I^2 index. The fixed effects model is used to calculate the 95% confidence interval (CI) of the average difference; if $I^2 \geq 50\%$, the random effects model is executed. Calculate the combined Odds Ratio (OR) (95% CI) to study the relationship between PROX1 expression and clinicopathological and prognostic parameters. The funnel chart is used to determine whether or not a publication is biased. A significant difference is defined as a p-value ≤ 0.05.

Results

Description of Studies

A total of 1289 patients in 8 articles were pooled in this meta-analysis (Fig. 1). We identified 65 articles from 9 database searches. 34 articles were duplicates and excluded. Among the rest of the whole 31 articles were screened for eligibility, 23 articles were excluded, including cell and animal experiments (N=7), review only (N = 4), other cancers (N = 5) and no clinical data (N = 7). The research comprised eight studies, all in line with the survey design. Patient cases are from five countries. Supporting Table 1 summarizes the extracted data from our included studies. Studies scoring 6 or more on the NOS.

Table 1
Main characteristics of the eligible studies.

No.	First author	Year	N.	Gender(M/F)	During	Country	Method	NOS
1	Abeer M. Hafez (13)	2021	50	37/13	2014-2019	Egypt	IHC	8
2	Zhu Li (20)	2021	110	64/46	2014-2015	China	IHC	6
3	Congcong Min (21)	2020	85	55/30	2013-2015	China	IHC	7
4	Aaro Kasurinen (11)	2019	275	135/140	2000-2009	Finland	IHC	8
5	KOJI UETA (16)	2018	99	75/24	2011-2012	Japan	IHC	7
6	Kang-Jin Park (15)	2017	327	215/112	1999-2000	Korea	IHC	6
7	Alli Laitinen (12)	2017	273	130/143	2000-2009	Finland	IHC	7
8	Wenan Wu (22)	2018	70	45/25	2010-2015	China	IHC	6

Expression of PROX1 and gender

A total of 1179 patients from 7 studies, including 692 males and 487 females, were pooled in the analysis. Existing meta-analyses found no difference in PROX1 expression between genders (OR: 1.234, 95%CI: 0.958-1.590, P = 0.104) (Fig. 2).

Expression of PROX1 and depth of tumor invasion

The meta-analysis included 1,024 patients from 5 studies to evaluate the correlation between tumor invasion depth (T1-T2 and T3-T4 groups) and PROX1 expression. PROX1 expression has no discernible relationship with tumor invasion depth (OR: 0.742, 95% CI: 0.428-1.287, P = 0.289) (Fig. 3).

Expression of PROX1 and lymph node metastasis

A total of 1210 GC patients from 6 studies reported the relationship between lymph node involvement and the expression of PROX1 (OR: 2.161, 95%CI: 0.808-5.779, P = 0.125) (Fig. 4). Meta-analysis showed that the expression of PROX1 has nothing to do with the presence or absence of lymph node metastasis.

Expression of PROX1 and TNM stage

We conducted 1176 patients from 7 studies in meta-analysis and showed that PROX1 expression was not related to the existence of TNM staging in GC (group I-II and group III-IV). (OR: 1.324, 95%CI: 0.572-

3.066, P = 0.513) (Fig. 5).

Expression of PROX1 and tumor size

We meta-analyzed 728 patients from four trials and discovered that PROX1 expression in GC patients is independent of tumor size (≥5cm and <5cm).(OR: 0.889, 95%CI: 0.502-1.576, P = 0.687) (Fig. 6).

Expression of PROX1 and metastasis:

858 patients were pooled from 6 studies and the meta-analysis indicates that PROX1 expression is unrelated to metastases in GC patients (OR: 1.096, 95%CI: 0.470-2.555, P= 0.763) (Fig. 7).

Expression of PROX1 and OS

A total of 1231 patients from 7 studies were combined to assess the relationship between the expression of PROX1 and OS in GC patients. The findings showed that expression of PROX1 was not linked to one year, three years, and five year OS of GC patients (one year OS: OR: 0.908, 95%CI: 0.631-1.306, P = 0.602; three years OS: OR: 1.234, 95%CI: 0.482-3.160, P = 0.661; five years OS: OR: 0.853, 95%CI: 0.266-2.736, P = 0.790) (Fig. 8).

The Cancer Genome Atlas Analysis

To conduct additional studies on the association between PROX1 expression and GC patients in terms of its prognostic value, we used the clinical data from TCGA and GTEx. The dataset includes 375 GC patients and 391 normal gastric control groups (Fig. 9). The contrast expressed that the expression of PROX1 was enhanced in GC patients (P<0.001). Moreover, 370 patients with GC were divided into the PROX1 group with high expression (G1; n = 187) and the PROX1 group with low expression (G2; n = 183). In comparison to individuals with low PROX1 expression, those with high PROX1 expression had a shorter OS. Nevertheless, the change was insignificant statistically (p=0.119). This conclusion supports our meta-analysis result, which further proves that expression of PROX1 was not associated with OS.

Discussion

Previous studies have explored the relationship between PROX1 expression and clinicopathological parameters and prognosis in various cancers. Currently, a growing number of studies investigate the relationship between the expression of PROX1 and GC. PROX1 could be a biomarker to diagnose the GC is seriously inconsistent. Our meta-analysis showed the following: (I) the expression of PROX1 has no relationship with clinicopathological parameters of GC, (II) the expression of PROX1 has no relationship with overall survival and (III) the expression of PROX1 cannot be used as a biomarker for diagnosing and preventing GC.

Some studies reported there are no relations between the expression of PROX1 and clinicopathological parameters in GC patients (11–12). Several studies indicated the correlation between the expression of PROX1 and depth of invasion, cancer stage and lymph node metastasis. But the results were controversial. Kang-Jin Park et al. reported a total of 327 patients finding that PROX1 expression was associated with lymph node metastases and cancer stage in a positive manner but no relation with the depth of invasion (15). They discovered that overexpression of PROX1 increased lymphatic endothelial cell invasion and tube formation by increasing VEGF-C and VEGF-D expression, which may result in tumor lymphangiogenesis. MiR-489 was shown to suppress the formation of GC through the HDAC7 and P13k/AKT pathways, was a direct target of PROX1, and showed a negative association with PROX1 protein expression (14) (23). However, Laitinen A et al. reported 273 patients in total to indicate the expression of PROX1 is negatively correlated with the depth of invasion, lymph node metastasis and cancer stage by their study (10). They also think that the mechanism research in the relationship between PROX1 expression and the presence of GC in patients is not comprehensive yet. In our meta-analysis, PROX1 expression was not related with gender, TNM stage, depth of invasion, tumor size, stage, tumor cell metastasis, or lymph node metastasis in GC patients.

Many studies have revealed the relationship between PROX1 expression and prognosis of tumor patients but the role is controversial. Several studies support the correlation between high expression of PROX1 and shorter OS in GC patients (15–16). But the other studies reported a total of 598 patients finding that PROX1 overexpression is associated with a longer OS (11–13). However, our meta-analysis reveals that PROX1 expression is not related with OS at 1, 3, or 5 years. A comprehensive genomics-based bioinformatic analysis study that including 375 cases with OS data for GC patients also supports our viewpoint, confirmed that expression of PROX1 was not associated with OS at 1, 3 or 5 years.

PROX1, a reliable and sensitive marker for endothelial lymph cells, is also a specific marker for the development of some organs in the early stage (17–19). Previous studies have shown that PROX1 acts as a tumor promoter in gastrointestinal cancer cells, suggesting that it may be a potential prognostic biomarker and a novel target for GC treatment. (16). According to Abeer M. Hafez et al. (13) and KOJI UETA et al. (16), PROX1 expression acts as a good prognostic predictor for GC patients. But Ueta K et al. reported that PROX1 may be a bad promising prognostic biomarker (9). Our result in a comprehensive meta-analysis found that expression of PROX1 was not correlated with any clinicopathological parameters and OS at one, three and five years so it fails to be a meaningful biomarker to prevent and diagnose the GC.

This meta-analysis also has its limitations. First, the included studies were published only in English and Chinese. Second, the number of included studies and the total number of patients are small. Additional laboratory studies and analysis on larger, well-defined patient cohorts are required to untangle the connection between PROX1 and GC. Third, the extraction cutoffs (positive/negative or high/low) of the expression of PROX1 in GC tissues are not totally same and the standard of PROX1 expression is subjective. In addition, some data are derived from estimates of survival curves, not individual patient

data. These may induce the heterogeneity or bias in our results. We will go on in-depth research when more studies about the relationship between PROX1 and GC in clinical parameters and OS emerge.

Conclusion

The current meta-analysis was conducted to determine the clinicopathological and prognostic relevance of the relationship between PROX1 expression and GC patients. Our meta-analysis report shows that the expression of PROX1 was not associated with gender, TNM stage, depth of invasion, tumor size, stage, tumor cell metastasis and lymph node metastasis. The expression of PROX1 was not associated with OS and it fails to be a meaningful biomarker to prevent and diagnose the GC.

Declarations

Availability of data and materials

The meta-analysis data generated or analyzed during this study is included in this published article and its supplementary information files. The datasets used and/or analyzed during the current study are available from TCGA repository: https://portal.gdc.cancer.gov/; GTEx repository: https://www.gtexportal.org/home/.

Author Contributions

Conceived and designed the experiments: GZ. Performed the experiments: ZJ, YW and JG. Analyzed the data: ZJ, YW and JG. Contributed reagents/materials/analysis tools: ZJ, YW and JG. Wrote the paper: ZJ, YW and JG. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Consent for publication

Not Applicable.

Ethics approval and consent to participate

Not applicable (this paper was provided based on researching in global databases).

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Figures

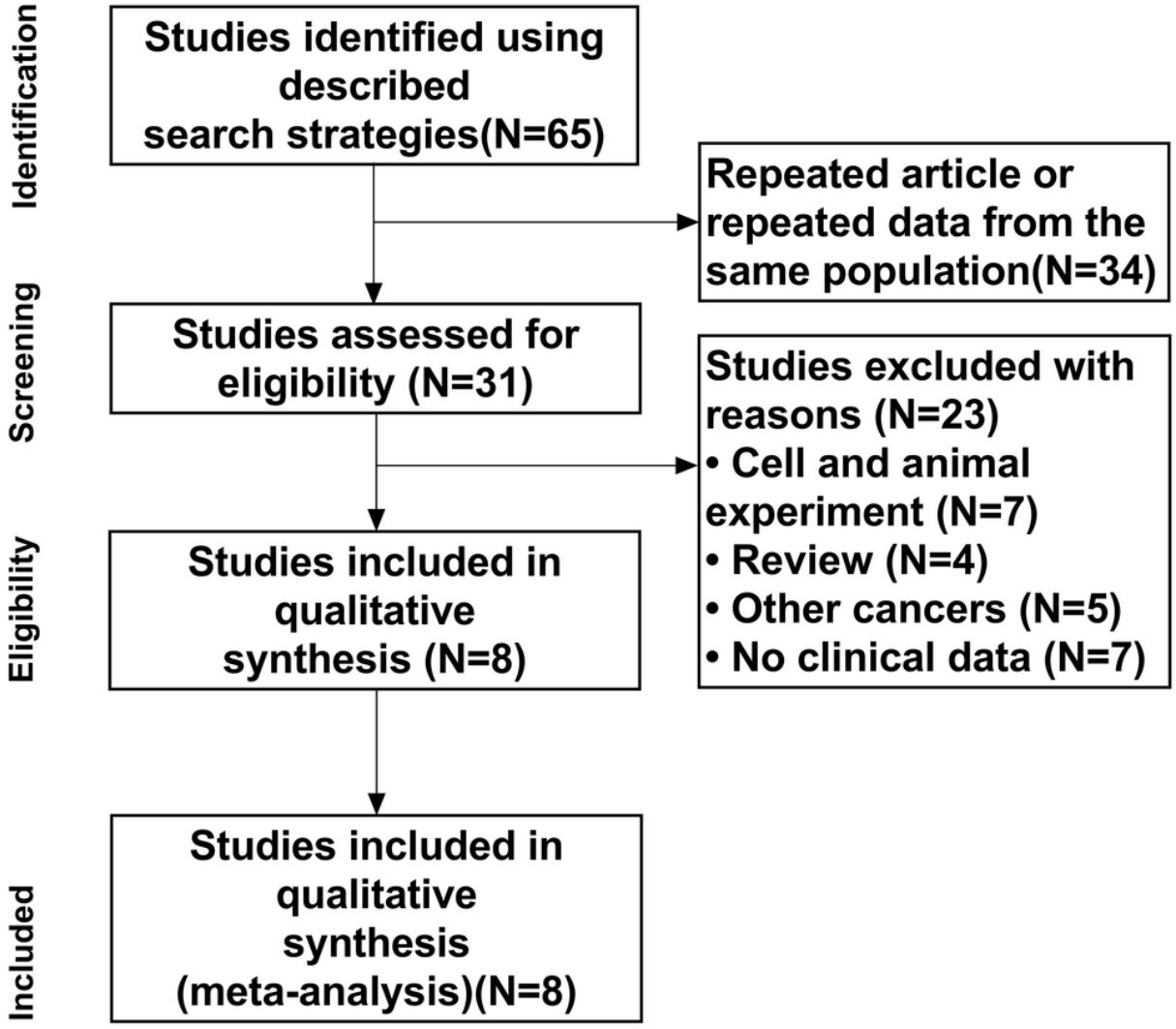

Figure 1

Flowchart for selection of studies

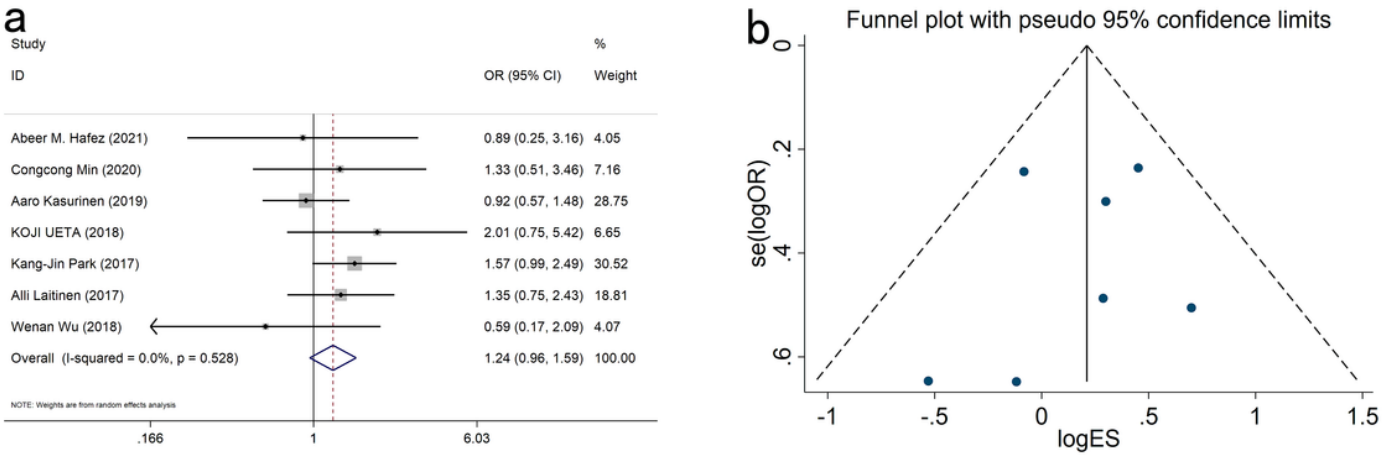

Figure 2

Forest plot(a) and funnel plot(b) for the relationship of PROX1 expression with gender

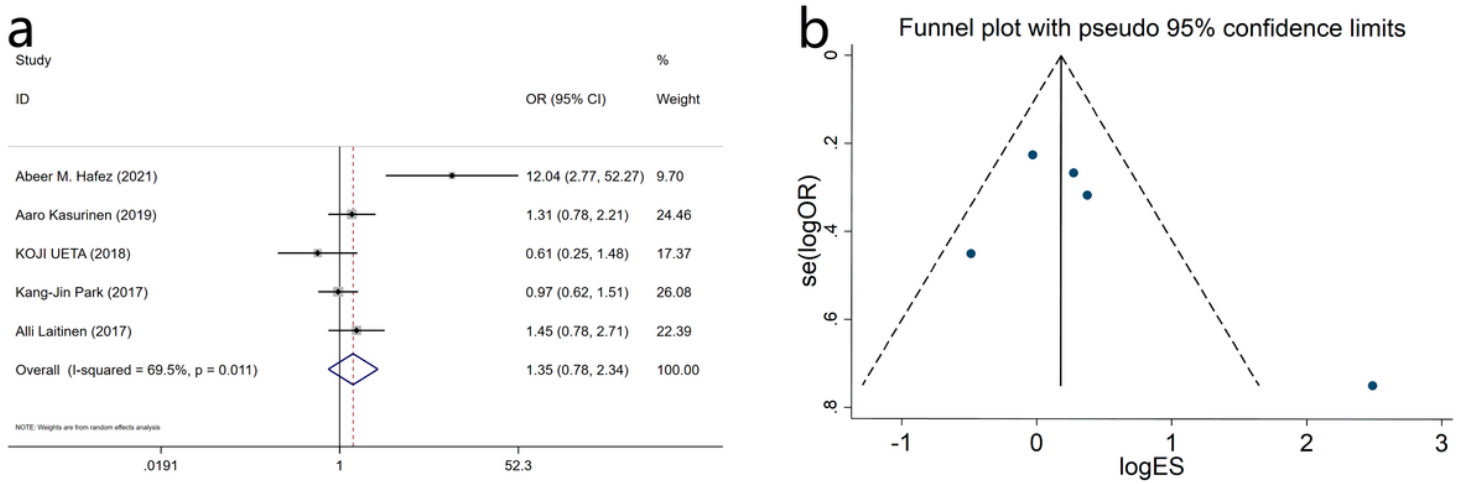

Figure 3

Forest plot(a) and funnel plot(b) for the relationship of PROX1 expression with the depth of tumor invasion

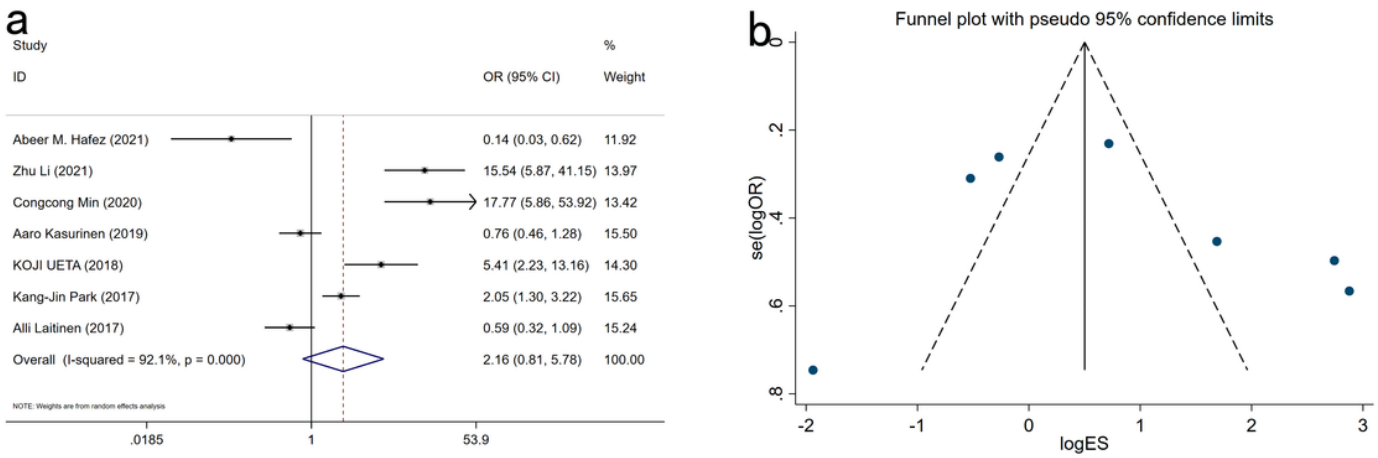

Figure 4

Forest plot(a) and funnel plot(b) for the relationship of PROX1 expression with lymph node metastasis

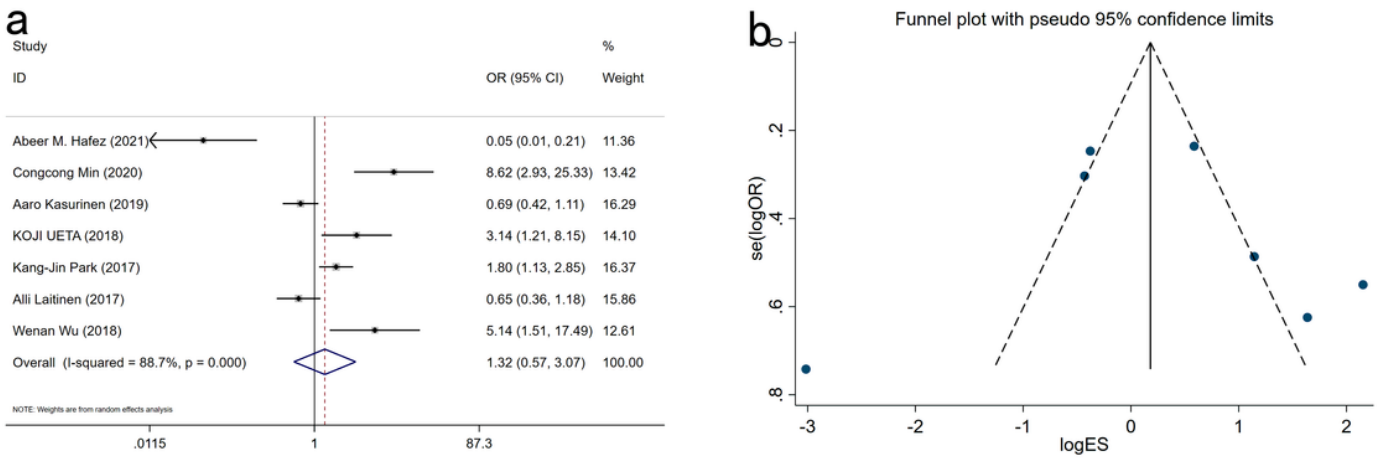

Figure 5

Forest plot(a) and funnel plot(b) for the relationship of PROX1 expression with TNM stage

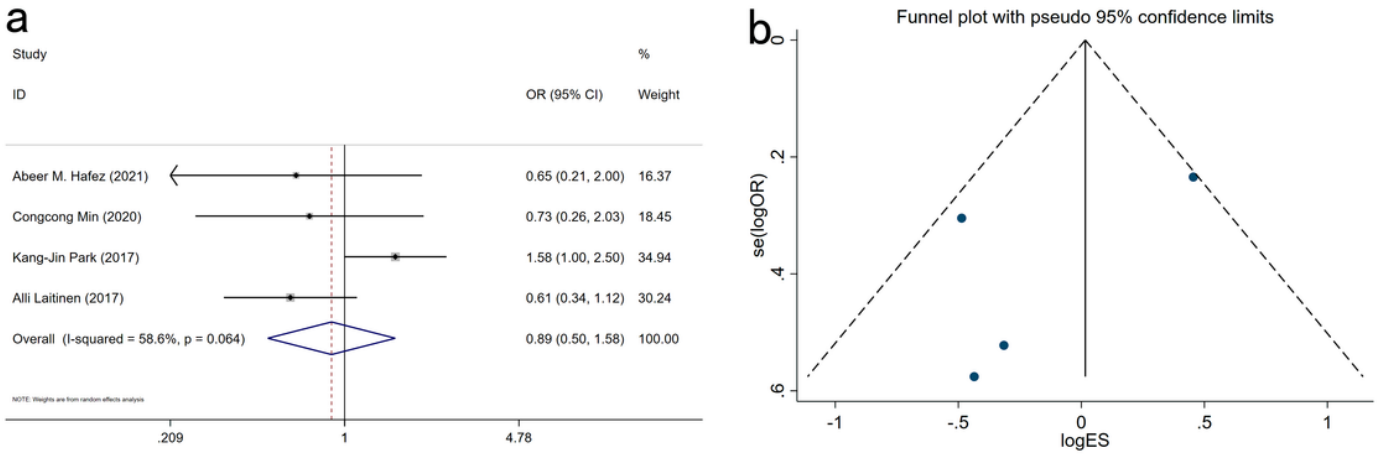

Figure 6

Forest plot(a) and funnel plot(b) for the relationship of PROX1 expression with tumor size

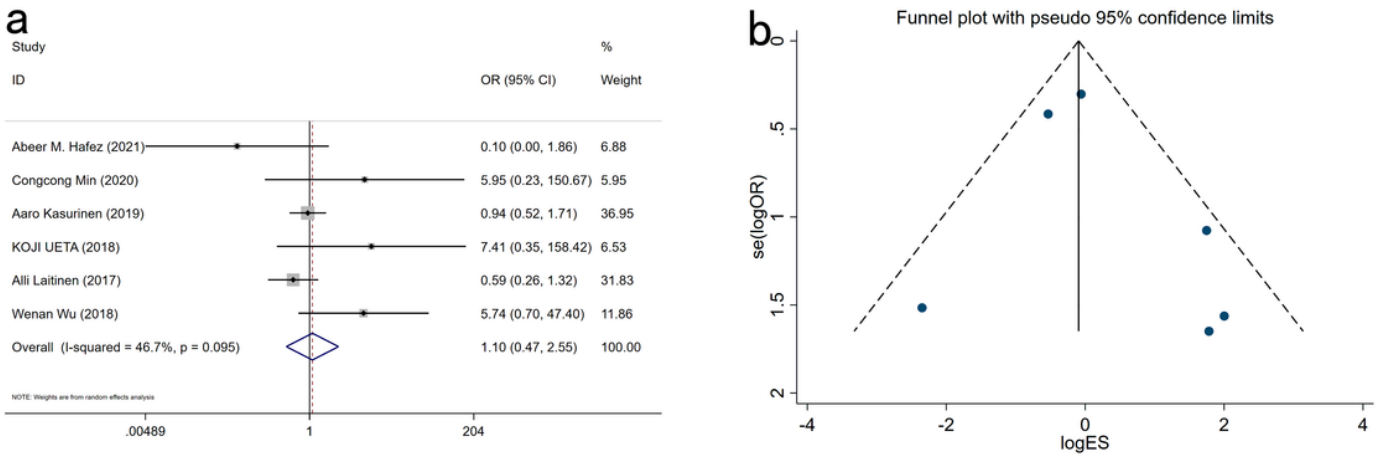

Figure 7

Forest plot(a) and funnel plot(b) for the relationship of PROX1 expression with metastasis

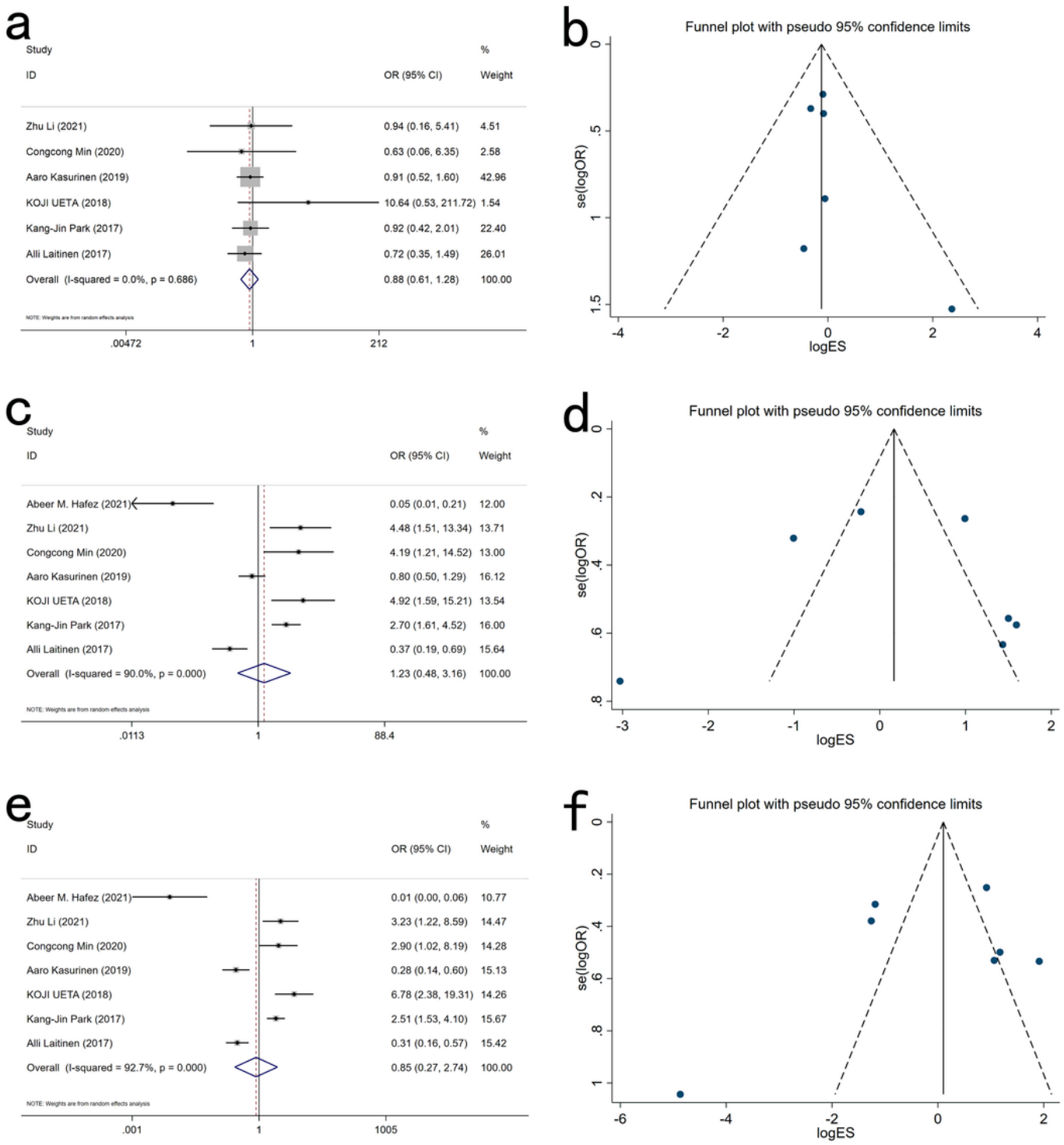

Figure 8

Forest plots for the association of PROX1 expression with OS in 1(a), 3(c) and 5(e) years and funnel plots for the association of PROX1 expression with OS in 1(b), 3(d) and 5(f) years

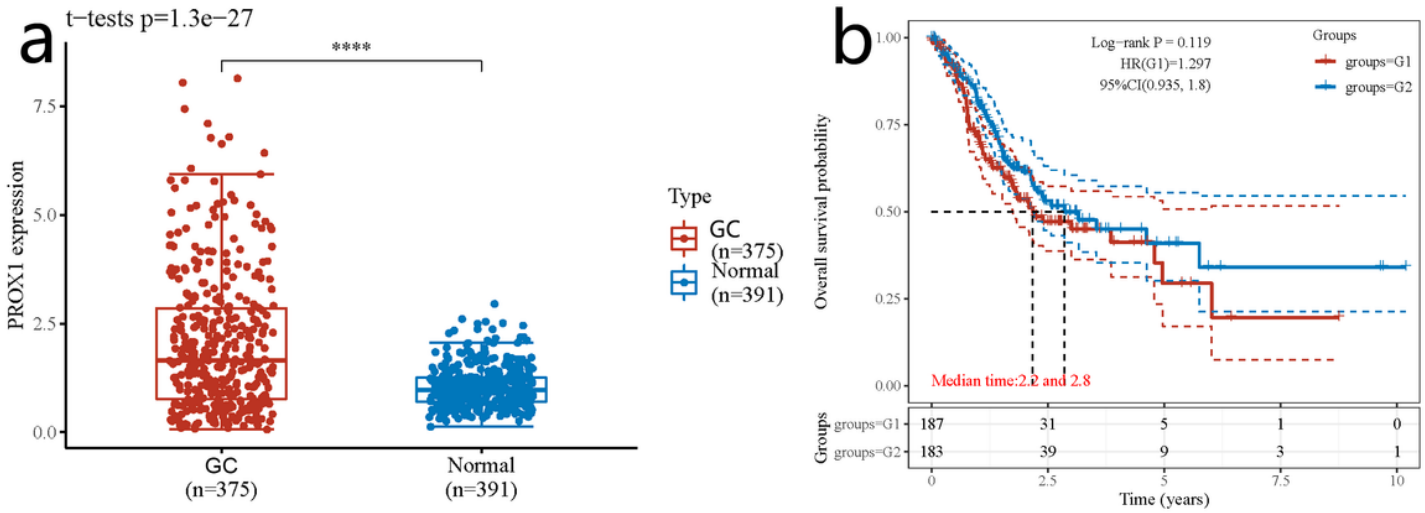

Figure 9

The relationship between PROX1 expression in gastric cancer (GC) patients and its prognostic value in The Cancer Genome Atlas (TCGA) cohort. (a) The amount of PROX1 expression in GC tissue versus normal gastric tissue ($p < 0.001$). (b) Overall survival (OS) plots of PROX1(G1: high-expression group, G2: low-expression group) in GC patients from TCGA cohort (log-rank $p = 0.119$). ****indicated that $P < 0.001$ compared with the GC group.