

# Prognostic role of targeting protein for Xklp2 in solid tumors

## A PRISMA-compliant systematic review and meta-analysis

Shijie Wang, MD, Yongyuan Chen, MD, Ying Chai, MD\*

### Abstract

**Background:** The prognostic role of targeting protein for Xklp2 (TPX2) in solid tumors has been investigated in several researches, but the results remain controversial. Here we present a meta-analysis to systematically review the association between TPX2 expression levels and prognosis of human solid tumors.

**Methods:** Studies published until December 2017 were searched in PubMed, Web of Science, and EBSCO, 13 studies (2134 patients) were collected for analysis. Odds ratios (ORs) for overall survival (OS) and disease-free survival (DFS) from individual studies were calculated by the application of Mantel-Haenszel random effect model. Pooled ORs were estimated by Z test. Publication bias and interstudy heterogeneity analyses were also performed.

**Results:** TPX2 overexpression was associated with poor OS at 3 and 5 years [OR=4.63, 95% confidence interval (CI): 3.27–6.56,  $P < .00001$ ; OR=4.05, 95% CI: 2.32–7.07,  $P < .00001$ , respectively] of solid tumors. Similar results were observed with DFS at 3 and 5 years (OR=3.35, 95% CI: 1.83–6.14,  $P < .0001$ ; OR=2.94, 95% CI: 1.74–4.98,  $P < .0001$ , respectively). Subgroup analysis revealed that increased TPX2 expression was related to worse prognosis of gastric cancer and hepatocellular cancer, while irrelevant to esophageal squamous cell cancer at 5-year survival rate.

**Conclusions:** Overexpression of TPX2 is related to poor survival rate in most solid tumors, which indicates that the expression level of TPX2 is a significant prognostic parameter and potential therapeutic target in various solid tumors.

**Abbreviations:** CI = confidence interval, DFS = disease-free survival, IHC = immunohistochemistry, MAP = microtubule-associated protein, MT = microtubule, NOS = Newcastle-Ottawa Scale, OR = odds ratio, OS = overall survival, PLK1 = polo-like kinase 1, TPX2 = targeting protein for Xklp2.

**Keywords:** disease-free survival, meta-analysis, overall survival, prognosis, solid tumors, targeting protein for Xklp2

### 1. Introduction

Targeting protein for Xklp2 (TPX2) is a proliferation-related protein first described by Heidebrecht et al,<sup>[1]</sup> who found a nuclear antigen roughly 100 kDa molecular mass exclusively expressed in S, M, and G2 phases, and named p100 at that time. Before long, Vernos' group discovered that this novel cell cycle-related protein played an essential role in the localization of GST-Xklp2-tail to microtubule (MT) minus ends.<sup>[2]</sup> Further studies indicated that TPX2 controlled MT nucleation, function, and coaction with other cell structures as an MT-associated protein (MAP), and improper expression of TPX2 lead to chromosomal

instability, caused centrosome amplification, and developed aneuploidy, which highly correlated with the occurrences and developments of various tumors.<sup>[3–6]</sup> More recently, researchers found TPX2 promoted the proliferation and migration of tumor cells by regulating Aurora-polo-like kinase 1 (PLK1) cascades, which worked as key signaling modules in mitosis.<sup>[7,8]</sup> As a result, TPX2/Aurora-PLK1 signaling might be a potential therapeutic target in malignant tumors.<sup>[9]</sup> Huang et al<sup>[10]</sup> reported TPX2 silencing mediated by the joint action of microvesicles and ultrasonic radiation significantly inhibited the progression of SKOV3 cells, indicating an effective gene therapy against ovarian cancer.

Several research groups have paid special attention to the relationship between TPX2 expression and various human tumors, strong research evidence revealed that in most human cancers, such as lung,<sup>[11]</sup> hepatic,<sup>[12]</sup> colon,<sup>[13]</sup> pancreatic,<sup>[14]</sup> salivary gland,<sup>[15]</sup> breast,<sup>[16]</sup> and cervical cancers,<sup>[17,18]</sup> TPX2 proved to be highly expressed. However, the prognostic value of the TPX2 expression in various solid tumors is still controversial.

Independent studies showed that in most cases overexpression of TPX2 had a negative impact on the prognosis of various types of solid tumors, such as astrocytoma,<sup>[19]</sup> ovarian cancer,<sup>[20]</sup> renal cell cancer,<sup>[21]</sup> gastric cancer,<sup>[22–24]</sup> bladder cancer,<sup>[25]</sup> esophageal squamous cell cancer,<sup>[26]</sup> hepatocellular cancer,<sup>[12,27]</sup> colon cancer,<sup>[28]</sup> and lung cancer.<sup>[29]</sup> However, Pan et al<sup>[30]</sup> found that the expression of TPX2 in tumor tissues of patients with prostate cancer was not related to their survival time.

Therefore, to clarify a better understanding of the prognostic significance of TPX2 overexpression in human solid tumors, we

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performed a meta-analysis combining 13 studies (2134 patients) as well as subgroups analysis, aiming to assess the correlation of elevated TPX2 expression with survival in solid tumors, and to learn a bit more about the clinical role of TPX2 as a therapeutic target and prognostic biomarker for solid tumors.

## 2. Methods

This meta-analysis was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.<sup>[31]</sup> This study is a summary and analysis of previous published studies, so there is no need for ethical approval.

### 2.1. Identification and selection of studies

We searched 3 different online databases, EBSCO, PubMed, and Web of Science, for studies that evaluated the relationship between TPX2 expression and survival in solid tumors until December 2017. The search terms included “Targeting protein for Xklp2” or “TPX2” or “DIL-2” or “C20orf2” and “neoplasms,” the search results were limited to the studies of human solid tumors. No publication time and language restrictions were imposed. Of the 3 databases, we identified

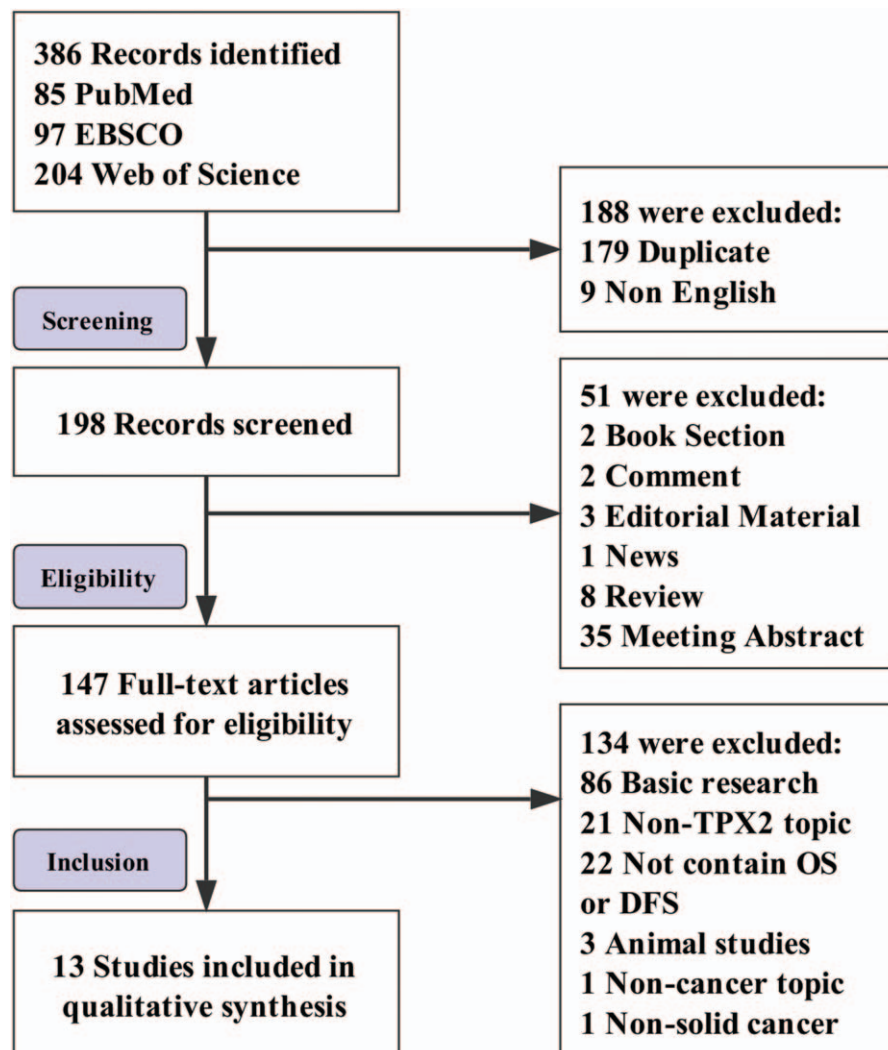
97, 85, and 204 items, respectively. The inclusion criteria were the literatures provided overall survival (OS) data or disease-free survival (DFS) data or both of them, follow-up time at least 3 years and writing in English. We manually filtered the retrieved articles to guarantee the sensitivity of the search strategy. Inter-rater reliability was evaluated by Cohen kappa coefficient. If there were disagreements, resolved through consensus.

### 2.2. Endpoints of interest

The primary endpoints were 3- or 5-year OS or DFS. Patients were assigned to control or experimental arms according to the TPX2 expression cut-off values defined by individual studies.

### 2.3. Data extraction

Two authors (SW and YC) independently reviewed the articles and extracted information using predesigned data form, which contained the publication time and first author of article, country of origin, tumor type, number of patients, age, histological type and stage, follow-up time, cut-off to determine TPX2 positivity, number of TPX2 positive patients and controls, detection method, and outcome endpoint. Survival data were extracted



**Figure 1.** Flow diagram of the meta-analysis process. DFS = disease-free survival, OS = overall survival.

**Table 1****Characteristics of studies included in the meta-analysis.**

Ref	Type of cancer	City, country	No.	Age, y	Male/Female	Stage	Follow-up, mo	Detection method	PTX2 (±) total No.	3-Year OS or DFS (±) %	5-Year OS or DFS (±)%	NOS
Studies including OS												
Bin Li et al (2010)	Malignant astrocytoma	Shanghai, China	52	NR	NR	I, II, III, and IV	NR	Immunohistochemistry	13/39	22.9/40.6	NA	6
Bo Liang et al (2016)	Gastric cancer	Nanchang, China	115	<55 59 ≥55 56	78/37	I, II, III, and IV	NR	Immunohistochemistry	54/61	27.6/70.3	NA	8
Cuijie Shao et al (2016)	Gastric carcinoma	Binzhou, China	106	53 (Range 28–75)	67/39	I, II, III, and IV	101 (Range 1–118)	Immunohistochemistry	71/35	13.2/74.4	7.4/49	8
Hung-Wei Pan et al (2017)	Prostate cancer	NR	467	NR	NR	Gleason score 6, 7, 8, 9, 10	NR	RNA-sequencing	276/191	97.3/98.4	95.9/92.7	5
Kaitia Y et al (2014)	Epithelial ovarian cancer	Montreal, Canada	131	62.6 (Range 34–87)	NR	I, II, III, and IV	37.2 (Range 1–142)	Immunohistochemistry	86/19	34.7/70.4	17.8/43.6	8
Liang Yan et al (2013)	Bladder carcinoma	Henan, China	71	<60 22 ≥60 49	46/25	T1, T2, T3, T4	60	Immunohistochemistry	59/12	44.9/83	1.7/50	7
Ping Wei et al (2013)	Colon cancer	Shanghai, China	203	<65 81 ≥65 122	86/117	I, II, III, and IV	NR	Immunohistochemistry	124/79	78.9/93.7	56.4/80.6	8
Po-Kuei Hsu et al (2014)-cDNA microarray cohort	Esophageal squamous cell carcinoma	Taiwan, China	16	62.6±13.8	15/1	T1, T2, T3, T4	27.5±30.2	cDNA microarray	7/7	14.3/42.9	14.3/28.6	8
Po-Kuei Hsu et al (2014), IHC cohort	Esophageal squamous cell carcinoma	Taiwan, China	97	61.0±12.5	90/7	I, II, III, and IV	25.0 (Range 21.21–28.80)	Immunohistochemistry	40/56	20/38.2	12.4/28.8	8
Qi Chen et al (2016)	Renal cell carcinoma	Xi'an, China	286	Males 61.1±16.3 females 62.5±17.4	192/94	I, II, III, and IV	72	Immunohistochemistry	179/56	38.8/70.5	26.8/65.7	8
Qingquan Liu et al (2015)	Hepatocellular carcinoma	Xi'an, China	130	52 (Range 33–76)	104/26	I, II, III, and IV	55 (Range, 3–79)	Immunohistochemistry	62/68	45.8/86.6	31.5/69.6	9
Ying Ma et al (2006)	Lung cancer	Beijing, China	82	NR	NR	I, II, III, and IV	60	Immunohistochemistry	48/34	24.6/70.5	16.7/58.8	6
Yuqi Huang et al (2014)	Hepatocellular cancer	Guangzhou, China	86	<50 27 ≥50 59	69/17	I, II, III, and IV	NR	Immunohistochemistry	56/30	31.6/56.1	19.1/46.2	7
Studies including DFS												
Chiharu Tomii et al (2017)	Gastric cancer	Tokyo, Japan	290	<65 129 ≥65 161	219/71	T1, T2, T3, T4	62 (Range, 2–111)	Immunohistochemistry	123/167	72/84.9	69.9/82.8	8
Po-Kuei Hsu et al (2014), cDNA microarray cohort	Esophageal squamous cell carcinoma	Taiwan, China	16	62.6±13.8	15/1	T1/2 4 T3/4 12	27.5±30.2	cDNA microarray	7/7	14.3/42.9	14.3/28.6	8
Po-Kuei Hsu et al (2014), IHC cohort	Esophageal squamous cell carcinoma	Taiwan, China	97	61.0±12.5	90/7	I, II, III, and IV	25.0 (Range 21.21–28.80)	Immunohistochemistry	40/56	12.4/33.3	12.4/29.2	8
Qingquan Liu et al (2015)	Hepatocellular carcinoma	Xi'an, China	130	52 (Range, 33–76)	104/26	I, II, and III	55 (Range, 3–79)	Immunohistochemistry	62/68	27.3/75	16.1/55.5	9
Yuqi Huang et al (2014)	Hepatocellular carcinoma	Guangzhou, China	86	<50 27 ≥50 59	69/17	I, II, III, and IV	NR	Immunohistochemistry	56/30	25.2/41.4	8.4/16.8	7

DFS = disease-free survival, IHC = immunohistochemistry, NOS = Newcastle-Ottawa Scale, NR = not reported, OS = overall survival.

**Table 2****Evaluation of human targeting protein for Xklp2 by different detection method in the selected studies.**

Ref	Type of cancer	Detection method	TPX2+ Tumor (%)	Cutoff for overexpression
Bin Li et al (2010)	Malignant astrocytoma	Immunohistochemistry	25.00	Immunoreactivity was classified according to the staining intensity and staining-positive cells proportion, and the overexpression of TPX2 was defined as strongly positive immunoreactivity (experimental group)
Bo Liang et al (2016)	Gastric cancer	Immunohistochemistry	46.96	Immunoreactivity was classified according to the overall staining index (OSI) by multiplying the scores of staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining) and staining-positive cells proportion (0, no staining; 1, <10% staining; 2, 10%–40% staining; or 3, >40% staining). The overexpression of TPX2 was defined as OSI >1 (experimental group)
Chiharu Tomii et al (2017)	Gastric cancer	Immunohistochemistry	42.41	<5% Stained cells (control group) vs ≥5% stained cells (experimental group)
Cuijie Shao et al (2016)	Gastric carcinoma	Immunohistochemistry	66.98	Immunoreactivity was classified according to the overall staining index (OSI) by multiplying the scores of staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining) and staining-positive cells proportion (0, no staining; 1, 0–10% staining; 2, 11–30% staining; 3, 31–70% staining; or 4, >71% staining). The overexpression of TPX2 was defined as OSI more than 0 (experimental group)
Hung-Wei Pan et al (2017)	Prostate cancer	RNA-sequencing	59.10	Overexpression of TPX2 was defined according to the cutoff value (6.8805) based on the 95th percentage expression level of 52 normal solid tissues
Katia Y et al (2014)	Epithelial ovarian cancer	Immunohistochemistry	81.90	Immunoreactivity was classified according to the staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining). The overexpression of TPX2 was defined as scores more than 0 (experimental group)
Liang Yan et al (2013)	Bladder carcinoma	Immunohistochemistry	83.10	Immunoreactivity was classified according to the overall staining index (OSI) by multiplying the scores of staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining) and staining-positive cells proportion (0, <5% staining; 1, 5%–25% staining; 2, 25%–50% staining; 3, 50%–75% staining; or 4, >75% staining). The overexpression of TPX2 was defined as OSI >0 (experimental group)
Ping Wei et al (2013)	Colon cancer	Immunohistochemistry	61.08	Immunoreactivity was classified according to the overall staining index (OSI) by adding up the scores of staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining) and staining-positive cells proportion (0, no staining; 1, <10% staining; 2, 10–50% staining; 3, >50% staining). The overexpression of TPX2 was defined as OSI more than 2 (experimental group)
Po-Kuei Hsu et al (2014), cDNA microarray cohort	Esophageal squamous cell carcinoma	cDNA microarray	50.00	Overexpression of TPX2 was defined according to the median expression level of 16 patients
Po-Kuei Hsu et al (2014), IHC cohort	Esophageal squamous cell carcinoma	Immunohistochemistry	41.67	Immunoreactivity was classified according to the overall staining index (OSI) by multiplying the scores of staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining) and staining-positive cells proportion (0, no staining; 1, 1%–25% staining; 2, 26%–75% staining; 3, >75% staining). The overexpression of TPX2 was defined as OSI more than median score (experimental group)
Qi Chen et al (2016)	Renal cell carcinoma	Immunohistochemistry	76.17	Immunoreactivity was classified according to the staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining). The overexpression of TPX2 was defined as scores more than 1 (experimental group)
Qingquan Liu et al (2015)	Hepatocellular carcinoma	Immunohistochemistry	47.69	Immunoreactivity was classified according to the overall staining index (OSI) by multiplying the scores of staining intensity (0 = no staining; 1 = weak staining; 2 = moderate staining; or 3 = strong staining) and staining-positive cells proportion (0, no staining; 1, 1%–10% staining; 2, 11%–50% staining; 3, 51%–80% staining; 4, >80% staining). The overexpression of TPX2 was defined as OSI more than median score (experimental group)
Ying Ma et al (2006)	Lung cancer	Immunohistochemistry	58.54	Immunoreactivity was classified according to the staining-positive cells proportion (0, no staining; 1, 1%–10% staining; 2, 11%–30% staining; 3, 31%–50% staining; 4, >50% staining). The overexpression of TPX2 was defined as scores >1 (experimental group)
Yueji Huang et al (2014)	Hepatocellular carcinoma	Immunohistochemistry	65.12	Immunoreactivity was classified according to the staining-positive cells proportion (0, no staining; 1, 1%–10% staining; 2, 11%–30% staining; 3, 31%–50% staining; 4, >50% staining). The overexpression of TPX2 was defined as scores >0 (experimental group)

IHC = immunohistochemistry, OS = overall survival, TPX2 = targeting protein for Xklp2.

from texts, tables, or Kaplan-Meier curves for both control (negative or low TPX2 expression) and experimental (positive or high TPX2 expression) arms. Some articles only provided Kaplan-Meier curves, in these cases, we extracted data points using Engauge Digitizer 10.4. For the studies included in our meta-analysis were all retrospective cohort studies, we assessed research quality by Newcastle-Ottawa Scale (NOS).<sup>[32,33]</sup> Researches with scores >6 points were considered to be high-quality researches.

**2.4. Statistical analysis**

The relative frequency of OS or DFS at 3 and 5 years between control and experimental arms was presented as an odds ratio (OR) with 95% confidence interval (CI) calculated by the application of Mantel-Haenszel random effect model. And the pooled ORs were estimated by Z test. Cochran Q and I<sup>2</sup> statistics were used to assess the heterogeneity. Subgroup analysis and sensitivity analysis were performed to explore the potential sources of heterogeneity. Sensitivity analyses were carried out for different detection methods and NOS scores of included studies. Subgroup analysis was performed on adequately sized subgroups (containing 2 researches or more). Publication bias was assessed by visual inspection of the funnel plot. All statistical analyses were performed using RevMan 5.3 analysis software (Cochrane Collaboration, Copenhagen, Denmark). All statistical tests were 2-tailed, and statistical significance was defined as P ≤ .05.

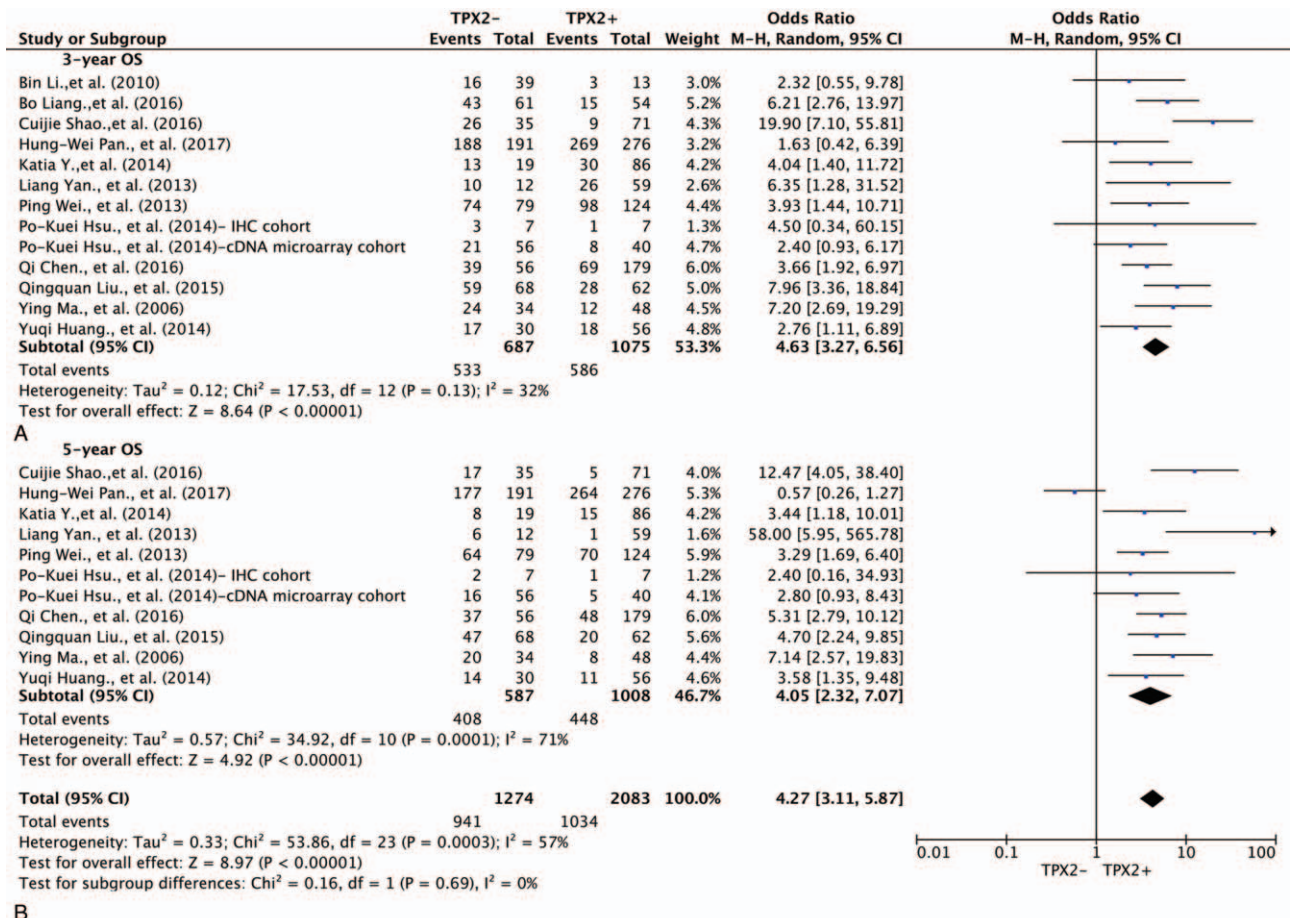
**3. Results**

**3.1. Search results and study characteristics**

A total of 386 records were retrieved from 3 databases by the initial search. After carefully reviewing, 13 studies with 2134 patients were finally included in our meta-analysis (Fig. 1). Characteristics of studies with OS or DFS data are presented in Table 1. Of these studies, 3 evaluated gastric cancer,<sup>[22-24]</sup> 2 evaluated hepatocellular cancer,<sup>[12,27]</sup> and 1 each evaluated malignant astrocytoma,<sup>[19]</sup> prostate cancer,<sup>[30]</sup> epithelial ovarian cancer,<sup>[20]</sup> bladder carcinoma,<sup>[25]</sup> colon cancer,<sup>[28]</sup> esophageal squamous cell cancer,<sup>[26]</sup> renal cell cancer,<sup>[21]</sup> and squamous cell lung carcinoma.<sup>[29]</sup> The quality scores of included studies varied from 5 to 9. Ten of 13 studies scored >6 points and considered to be high-quality researches (see Table, Supplemental Content, <http://links.lww.com/MD/C585>, which illustrates the quality scores of enrolled studies by Newcastle-Ottawa Quality Assessment).

**3.2. Evaluation and expression of TPX2**

Table 2 presents the TPX2 detection methods and cut-off values for TPX2 overexpression defined in the individual studies. Eleven investigations detected the TPX2 status by immunohistochemistry (IHC), 1 study used RNA-Sequencing, and the remaining 1 research used 2 detection methods, IHC, and cDNA microarray, respectively. For the IHC method, the cut-off points for TPX2



**Figure 2.** Forest plots showing odds ratios of TPX2- versus TPX2+ of all patients with solid tumors for OS at 3 and 5 years. A, 3-year OS. B, 5-year OS. CI = confidence interval, OS = overall survival, TPX2 = targeting protein for Xkpl2.

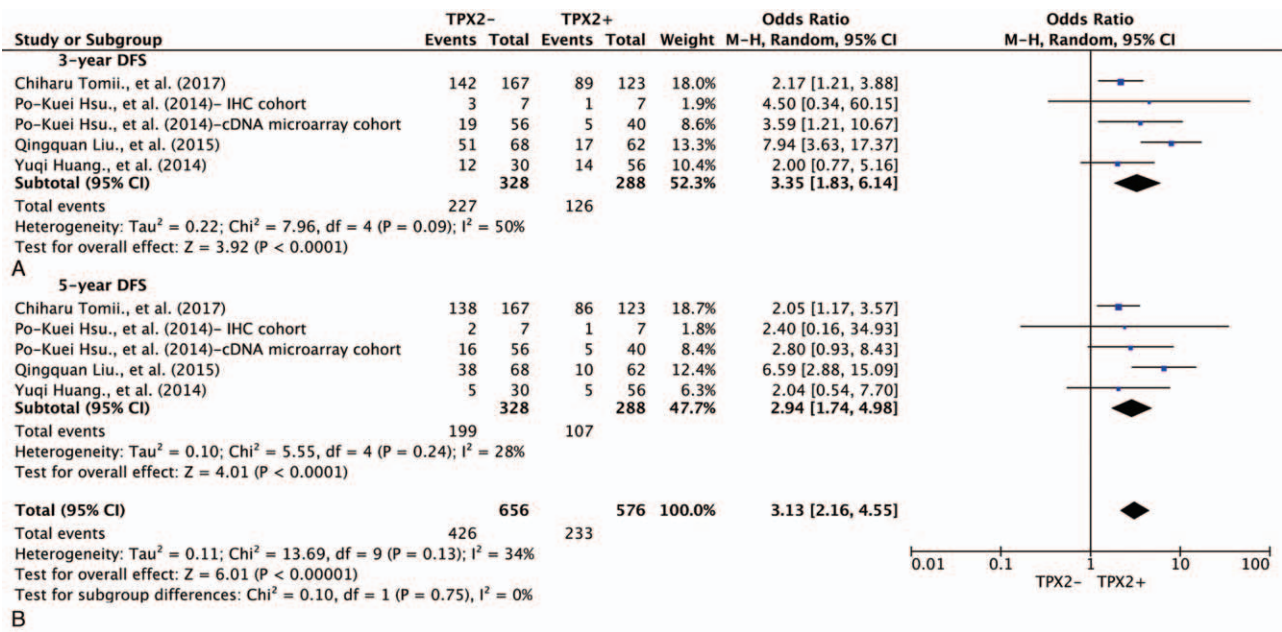


Figure 3. Forest plots showing odds ratios of TPX2- versus TPX2+ of all patients with solid tumors for DFS at 3 and 5 years. A. 3-year DFS; B. 5-year DFS. CI = confidence interval, DFS = disease-free survival, TPX2 = targeting protein for Xklp2.

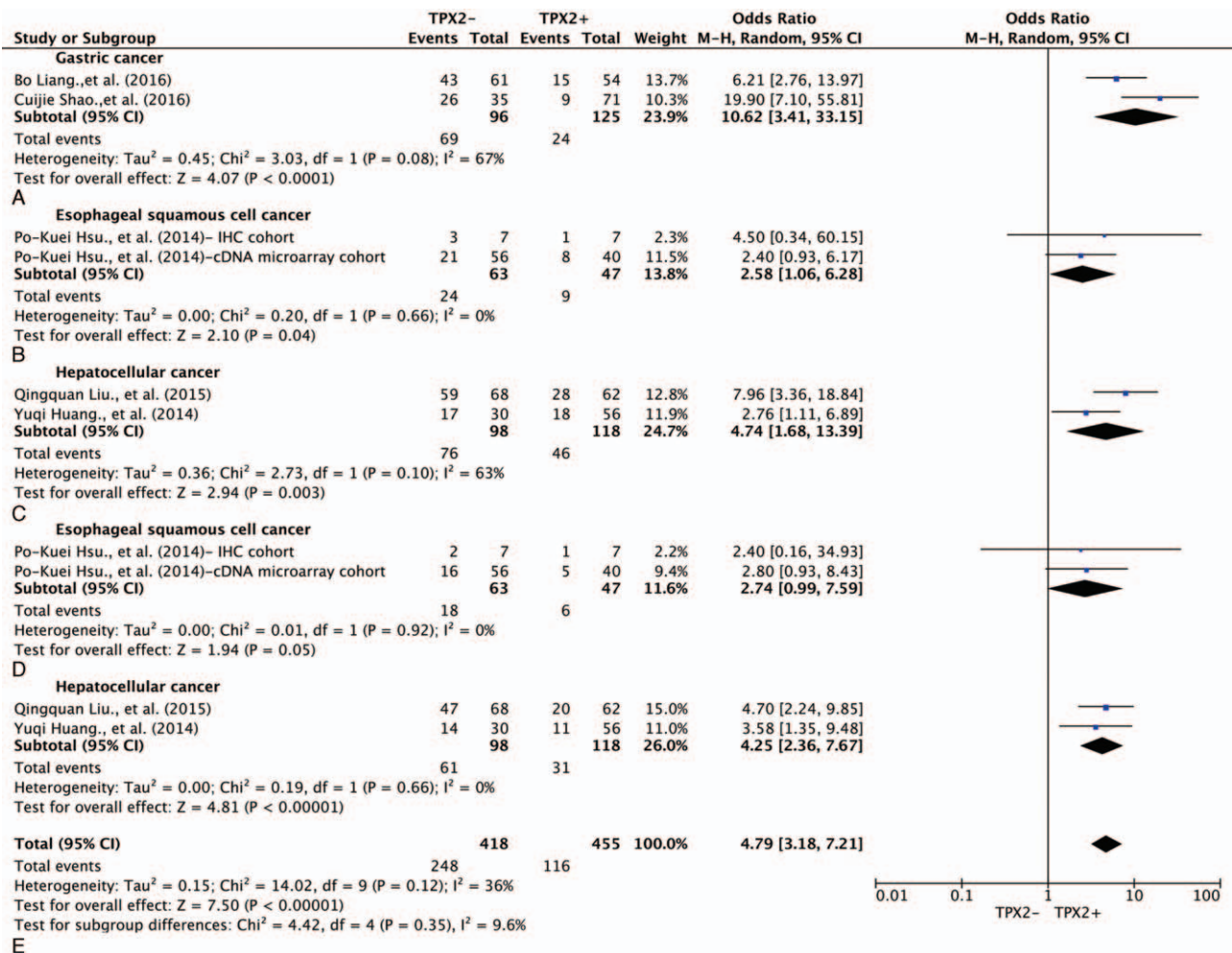


Figure 4. Subgroup analysis of overall survival (OS) at 3 and 5 years by the expression level of TPX2 in different cancer types. A, Gastric cancer and 3-year OS. B, Esophageal squamous cell cancer and 3-year OS. C, Hepatocellular cancer and 3-year OS. D, Esophageal squamous cell cancer and 5-year OS. E, Hepatocellular cancer and 5-year OS. CI = confidence interval, TPX2 = targeting protein for Xklp2.

positive or high expression according to the combination of masculine cells proportion and the staining intensity.

### 3.3. Association of TPX2 with survival

There were a total of 12 studies provided the OS data at 3 years, and 10 studies at 5 years. Results showed that TPX2 expression increased in tumor tissue had a negative effect to 3-year OS (OR=4.63, 95% CI: 3.27–6.56,  $P < .00001$ ), and 5-year OS (OR=4.05, 95% CI: 2.32–7.07,  $P < .00001$ ) (Fig. 2). Similarly, 4 studies reported survival data for both 3 and 5-year DFS, results showed that elevated TPX2 expression was also related with obviously poor 3-year DFS (OR=3.35, 95% CI: 1.83–6.14,  $P < .0001$ ), and 5-year DFS (OR=2.94, 95% CI: 1.74–4.98,  $P < .0001$ ) of solid cancers (Fig. 3). There was low or moderate heterogeneity of the data among studies. Thus, we performed subgroup meta-analysis to figure out whether the various cancer types lead to the heterogeneity.

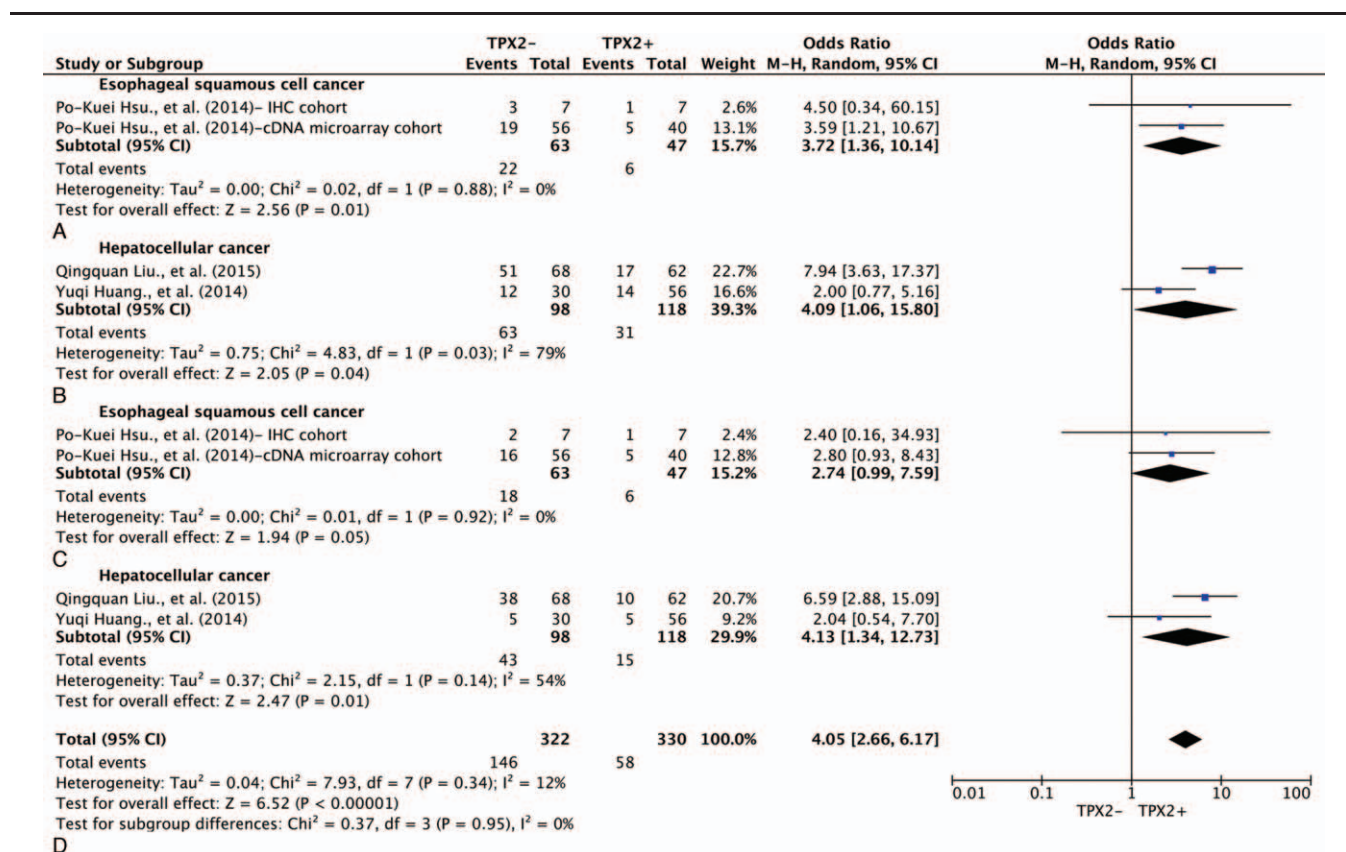
In the following analysis of subgroups classified by different cancer types, we discovered that TPX2 expression was related to the worse 3-year OS of gastric cancer (n=2, OR=10.63, 95% CI: 3.41–33.15,  $P < .0001$ ) (Fig. 4A), esophageal squamous cell cancer (n=2, OR=2.58, 95% CI: 1.06–6.28,  $P = .04$ ) (Fig. 4B), and hepatocellular cancer (n=2, OR=4.74, 95% CI=1.68–13.39,  $P = .003$ ) (Fig. 4C). Consistent with the results above, TPX2 expression was related to the worse 5-year OS of hepatocellular cancer (n=2, OR=4.25, 95% CI: 2.36–7.67,  $P < .00001$ ) (Fig. 4E). Also, we found TPX2 expression had a

negative relationship with 3-year DFS of esophageal squamous cell cancer (n=2, OR=3.72, 95% CI: 1.36–10.14,  $P = .01$ ) (Fig. 5A) and hepatocellular cancer (n=2, OR=4.09, 95% CI: 1.06–15.80,  $P = .04$ ) (Fig. 5B), and with 5-year DFS of hepatocellular cancer (n=2, OR=4.13, 95% CI=1.34–12.73,  $P = .01$ ) (Fig. 5D). However, significant relationship between TPX2 expression and 5-year OS/DFS of esophageal squamous cell carcinoma was not found (both  $P = .05$ ) (Figs. 4D and 5C).

### 3.4. Sensitivity analyses

Removing the researches with NOS score <7 did not affect results for OS at 3 or 5 years (OR=4.93, 95% CI: 3.35–7.24,  $P < .00001$ ; OR=4.63, 95% CI: 3.19–6.74,  $P < .00001$ , respectively), and did not affect results for DFS at 3 or 5 years (OR=3.88, 95% CI: 1.85–8.15,  $P = .0003$ ; OR=3.16, 95% CI: 1.65–6.05,  $P = .0005$ , respectively) as well. Elimination of these studies did not reduce heterogeneity for 3-year OS, 3-year DFS, or 5-year DFS (Cochran  $Q P = .14$ ,  $I^2 = 34\%$ ; Cochran  $Q P = .08$ ,  $I^2 = 56\%$ ; Cochran  $Q P = .15$ ,  $I^2 = 43\%$ , respectively), but reduce heterogeneity for 5-year OS (Cochran  $Q P = .23$ ,  $I^2 = 24\%$ ).

Removing the studies that TPX2 expression was not detected by IHC did not affect results for OS at 3 or 5 years (OR=4.91, 95% CI: 3.43–7.04,  $P < .00001$ ; OR=4.90, 95% CI: 3.41–7.04,  $P < .00001$ , respectively), and did not affect results for DFS at 3 or 5 years (OR=3.31, 95% CI: 1.70–6.46,  $P = .0004$ ; OR=2.99, 95% CI: 1.63–5.49,  $P = .0004$ , respectively) as well. Exclusion of these studies did not reduce heterogeneity for 3-year OS, 3-year



**Figure 5.** Subgroup analysis of disease-free survival (DFS) at 3 and 5 years by the expression level of TPX2 in different cancer types. A, Esophageal squamous cell cancer and 3-year overall survival (OS). B, Hepatocellular cancer and 3-year OS. C, Esophageal squamous cell cancer and 5-year OS. D, Hepatocellular cancer and 5-year OS. CI = confidence interval, TPX2 = targeting protein for Xklp2.

DFS or 5-year DFS (Cochran  $Q$   $P$  = .13,  $I^2$  = 34%; Cochran  $Q$   $P$  = .05,  $I^2$  = 62%; Cochran  $Q$   $P$  = .14,  $I^2$  = 46%, respectively), but reduce heterogeneity for 5-year OS (Cochran  $Q$   $P$  = .21,  $I^2$  = 27%).

### 3.5. Publication bias

Funnel plot analysis was performed to estimate the potential bias in the included publications, and results revealed that there was no significant publication bias for OS and DFS in our meta-analysis (Fig. 6).

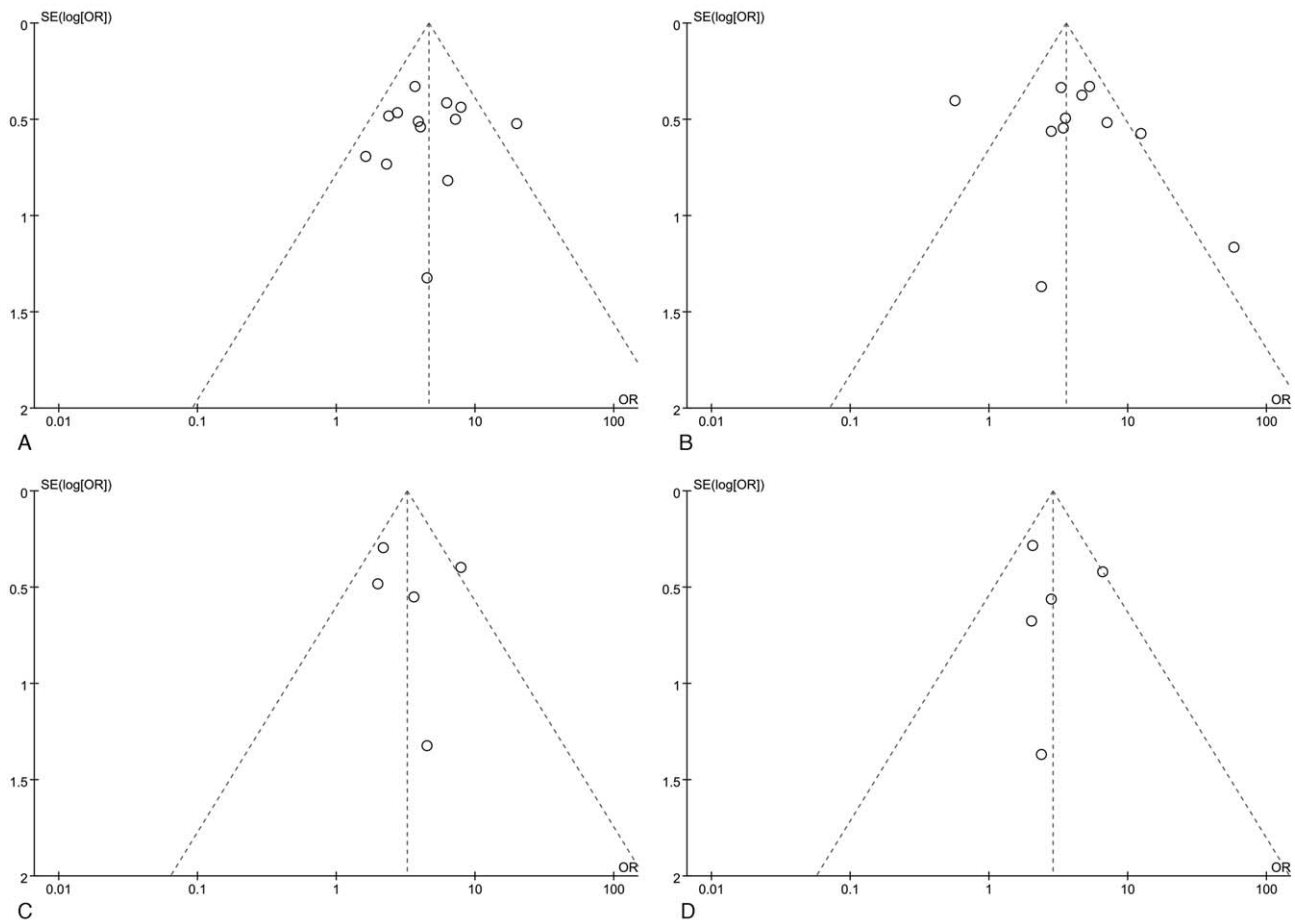
## 4. Discussion

The initiation and progression of tumors is a complex process with many factors may make a contribution, among them is the dysfunction of MT.<sup>[34]</sup> TPX2 is a kind of MAP controls MT function and dynamics, and therefore may play an important role in the development of tumors.

Recently, a systematic review containing 10 studies with 906 patients was carried out by Gang et al. Results showed that TPX2 was overexpression in most digestive system tumors and their expression status was associated with obviously worse survival rate.<sup>[35]</sup> In addition to digestive system tumors, TPX2 overexpression also exists in many other kinds of malignant

neoplasms. Most studies suggest that enhanced expression of TPX2 in tumor tissue worsens the clinical outcome and decreasing TPX2 levels may be a beneficial approach for cancer treatment. For instance, Yan et al found that transfection of TPX2 siRNA decreased the viability and proliferation capacity of bladder carcinoma cell lines and TPX2-depleted tumor cells obviously grew more slowly in nude mice.<sup>[25]</sup> However, Pan et al suggested that the TPX2 expression level was not significantly different in relation to cumulative survival in human prostate cancer patients.<sup>[30]</sup> In light of the significant role of TPX2 in clinical application, we performed this meta-analysis to evaluate the relationship between TPX2 expression and prognosis of patients with various solid tumors.

In this systematic analysis, we pooled and evaluated survival data from 13 studies, which including 2134 patients, and demonstrated that the elevated expression of TPX2 was a prognostic marker of unfavorable clinical outcome, with consistent results of OS or DFS at 3 and 5 years. In the subgroup analysis stratified by tumor types, elevated TPX2 expression was associated with unfavorable 3- and 5-year OS/DFS of hepatocellular cancer and gastric cancer. And though overexpression of TPX2 was related to worse 3-year OS/DFS of esophageal squamous cell cancer, no significant relationship between TPX2 expression and 5-year OS/DFS of esophageal squamous cell



**Figure 6.** A, Publication bias funnel plot of the studies assessing targeting protein for XKlp2 (TPX2) expression and 3-year overall survival in solid tumors. B, Publication bias funnel plot of the studies assessing TPX2 expression and 5-year overall survival in solid tumors. C, Publication bias funnel plot of the studies assessing TPX2 expression and 3-year disease-free survival in solid tumors. D, Publication bias funnel plot of the studies assessing TPX2 expression and 5-year disease-free survival in solid tumors. Visual inspection of the funnel plot did not identify substantial asymmetry.



cancer was found. Moreover, 1 study showed that TPX2 overexpression in prostate cancer tissues was associated with preferable 5-year OS. As far as we know, TPX2 is a protein with several functional regions. Its N-terminus directly binds to and activates Aurora A kinase, which is a potential cancer marker important for cell proliferation.<sup>[36]</sup> And its C-terminus binds to Eg5 and Xklp2 kinesin (named “targeting protein for Xklp2” for that reason), mediating these motors’ spindle localization.<sup>[3,37]</sup> Except for these, unfortunately, we know little about the molecular mechanism and function of TPX2 in cancer pathogenesis, and cannot explain why TPX2 plays a catalytic role in some tumors, but not in others. These discrepancies suggest further attention should be paid to discover how TPX2 works at the cellular and molecular levels.

What is novel about this research is that it is the first systematic evaluation of the literatures with respect to TPX2 expression and clinical outcomes in patients with various solid tumors. This research also involves several meaningful implications. Firstly, it shows that in most solid tumors, TPX2 overexpression is correlated with unfavorable outcomes. Thus, along with imaging technology and other tumor biomarkers,<sup>[38–40]</sup> TPX2 may play an essential role in assessing the prognosis of various neoplasms. Secondly, by subgroup analysis, TPX2 expression presents negative relation with outcomes in gastric cancer and hepatocellular cancer, but irrelevant to long-term outcome in esophageal squamous cell cancer, which indicated that the function of TPX2 may be heterogeneous in different tumors.

There are also several limitations presented in our meta-analysis. First, some survival data were extracted from Kaplan-Meier curves artificially; thus, slight deviations may exist, although they could hardly influence the analysis results. Second, the detection methods and cut-off values of TPX2 expression are nonuniform in different articles. Third, stratification analysis for most types of tumor failed to be performed, because there were not enough data so far. Lastly, most researches included in our meta-analysis were performed in China. Thus, no firm conclusions can be drawn in other kinds of people and the differences between human races are uncertain. Further studies need to be performed to confirm the impact of human species on the results of studies.

In conclusion, our meta-analysis demonstrates that TPX2 overexpression is related to unfavorable outcome in most solid tumors, implying that TPX2 is a potential prognostic marker and therapeutic target for various solid tumors.

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