Int J Clin Exp Med 2018;11(2):431-444
www.ijcem.com /ISSN:1940-5901/IJCEM0054037

Review Article

The prognostic value of Skp2 expression level in cancer: a meta-analysis

Jing Jia[1], Juan Ren[1], Yihui He[3], Zhenyu Jia[4], Shenglin Ma[2,5], Xiaoju Wang[1,2], Xiaoming Sun[6]

[1]Center for Molecular Medicine, [2]Institute of Lung Cancer, Zhejiang Academy of Medical Sciences, Zhejiang Academy of Medical Sciences, Hangzhou, 310013 Zhejiang, P.R. China; [3]Institute of Viral Disease, Zhejiang Academy of Medical Sciences, Hangzhou, 310013 Zhejiang, P.R. China; [4]Institute of Occupational Diseases, Zhejiang Academy of Medical Sciences, Hangzhou, 310013 Zhejiang, P.R. China; [5]Department of Radiation Oncology, Affiliated Hangzhou Hospital of Nanjing Medical University, Hangzhou, Zhejiang, P.R. China; [6]Department of Forensic Medicine, Xuzhou Medical College, Xuzhou, 221002 Jiangsu, P.R. China

Received March 28, 2017; Accepted August 15, 2017; Epub February 15, 2018; Published February 28, 2018

Abstract: Purpose: S-phase kinase-associated protein 2 (Skp2) is overexpressed in many types of cancers and might be a potential cancer prognostic biomarker. Several studies reported an association between Skp2 expression level and tumor prognosis; however, the results were inconsistent and inconclusive. The present study is a meta-analysis carried out to assess the association between Skp2 expression and tumor prognosis in accordance with the guidelines of the Preferred Reporting Item for Systematic Reviews and Meta-analyses. Methods: Until April 30, 2016, 35 articles were collected, representing 5,514 patients. Hazard ratio (HR) and 95% confidence intervals (CIs) were used to assess the association between Skp2 expression and survival outcome and estimated using fixed- or random-effects models when appropriate. The statistical significance of the pooled HR was determined by a Z-test. The statistical heterogeneity within studies was detected with the Chi-squared based Q-test and I^2 metric. The publication bias was also assessed by Egger's and Begg's tests. Sensitivity analysis was also carried out. STATA version 11.0 was used for all statistical analyses. Results: Results suggested that Skp2 overexpression is associated with poor overall survival (OS) and DFS/RFS in all cancer patients as well as in subgroup analysis. Skp2 localized in the cytoplasm was not associated with prognosis. Conclusion: Skp2 might be a predictive and independent marker of cancer prognosis.

Keywords: Skp2, tumor prognosis, meta-analysis, HR

Introduction

The prediction of prognosis for cancer treatment is under intensive investigation. Traditional biomarkers are often ineffective to assess prognosis and design treatment, and most cancer patients die from complications of advanced cancer after the first treatment rather than from the primary tumor diagnosis. Therefore, an effective method for early prediction of recurrence and survival in cancer patients is urgently needed. Proteins involved in tumor progression and metastasis might be potential biomarkers for cancer prediction. Until now, numerous biomarkers have been evaluated for cancer prognosis by various research groups; however, effective tumor prognosis biomarkers are still lacking.

S-phase kinase-associated protein 2 (Skp2), a member of the F-box protein family, is the substrate recognition subunit of Skp1-Cullin-F box protein (SCF) E3 ubiquitin ligase complex. Skp2 can recognize phosphorylated substrate proteins and mediate their ubiquitination by the SCF complex. Many proteins involved in tumor suppression can be degraded via Skp2-mediated proteolysis, so the *Skp2* gene is considered an oncogene. Skp2 displays its oncogenic activities by regulating cell cycle progression, senescence, and tumor metastasis [1, 2]. For example, the cell cycle inhibitors $p21_{Cip1/WAF}$ and $p27_{Kip1}$ can be targeted by Skp2 and further promote G1/S transition, causing tumor cell cycle progression [3]. Overexpression of Skp2 protein has been observed in many cancers and correlates with tumorigenesis as

well as with poor prognosis in a variety of human cancers, including prostate cancer[4], gastric cancer [5], breast cancer [6], and liver cancer [7].

Several studies have explored the association between Skp2 expression level and tumor prognosis [8-10]; however, the results were inconsistent or inconclusive, and limited by retrospective design or limited tumor type. Therefore, we carried out a meta-analysis to estimate the association between Skp2 expression and tumor prognosis. The heterogeneity between the individual studies as well as the existence of potential publication bias was also investigated.

Materials and methods

This meta-analysis was executed in accordance with the guidelines of the Preferred Reporting Item for Systematic Reviews and Meta-analyses.

Literature search

Studies were retrieved from the PubMed and Embase electronic databases. The search terms were "Skp2", "S-phase kinase-associated protein 2", "tumor (s)", "cancer (s)", "survival", "prognostic", and "prognosis". The last search ended on April 30, 2016. Additional eligible studies were identified by manual searches of articles referenced in the publications retrieved.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) the correlation between Skp2 expression and overall survival (OS), disease-specific survival (DSS), recurrence-free survival (RFS), or metastasis-free survival (MFS) was estimated; (2) Skp2 expression was evaluated; (3) a cohort design was used; (4) the hazard ratio (HR) and 95% confidence interval (CI) were available or a Kaplan-Meier curve was available from which the HR and 95% CI could be extracted; (5) the article was published in English. The exclusion criteria were as follows: (1) reviews, conference abstracts, editorials, or letters; (2) insufficient published data for estimating HR and 95% CI; (3) language other than English. When multiple publications were reported by the same group on a similar patient cohort, we included only the study with the largest number of patients.

Data extraction

Two investigators (Jing Jia and Xiaoming Sun) independently extracted the following data: first author's name, year of publication, patient ethnicity, cancer type, sample size, test method, antibody and dilution, cut-off value, and number of positive samples. In a single publication, when several analyses were carried out with different parameters, these analyses were defined as independent studies. As a result, the number of studies included was greater than the number of publications included. When the prognosis was plotted as a Kaplan-Meier curve, the HR digitizer Engauge 4.0 software (http://engauge-digitizer.software.informer.com/) was used to extract the data. A third investigator (Juan Ren) checked the data and solved inconsistencies through discussion.

Quality assessment

The quality of the publications included was assessed using the Newcastle-Ottawa Scale (NOS) (Wells GA) and ranked by a "☆" rating system. The score of each publication was the number of total "☆". The assessment was conducted by all of the authors. Studies with a score ≥ 7 were considered to be of high quality.

Statistical analysis

HRs and the corresponding 95% CIs were used to assess the association between Skp2 expression and survival outcome. The statistical significance of the pooled HR was determined by a *Z*-test $P < 0.05$ was considered to be statistically significant. The statistical heterogeneity within studies was detected with the Chi-squared based Q-test and I^2 metric (0%-25%: no heterogeneity; 25%-50%: moderate heterogeneity; 50%-75%: large heterogeneity; 75%-100%: extreme heterogeneity). When $P > 0.10$ or $I^2 < 50\%$, the fixed-effects model was used. In the opposite case, the random-effects model was used. The publication bias was also assessed by Egger's and Begg's tests, and the potential publication bias was considered significant if $P < 0.05$. Sensitivity analysis was also carried out. STATA version 11.0 (STATA Corporation, College Station, TX, USA) was used for all statistical analyses. All statistical tests were two-sided.

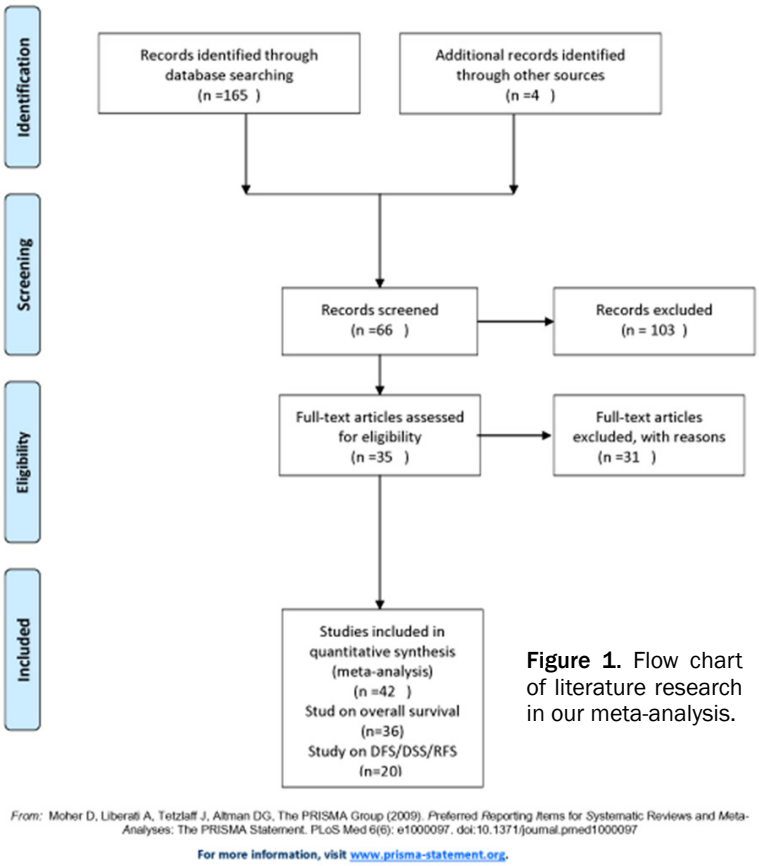

Figure 1. Flow chart of literature research in our meta-analysis.

Results

Eligible studies

In total, 169 relevant articles were identified (**Figure 1**). After an initial screening of the titles and abstracts, 103 publications were excluded for missing any assessment of the association between Skp2 expression and survival. The remaining 66 publications were carefully reviewed using the inclusion and exclusion criteria. Finally, 35 publications were selected for this meta-analysis, with a total of 5,514 patients [4, 5, 7, 8, 10-39]. Ethical approval and informed patient consent were not required since this study was a literature review with no direct patient contact or influence on patient care.

The main characteristics of the publications reviewed are listed in **Table 1**. The impact of Skp2 expression on OS was investigated in 27 publications. The effect of Skp2 expression on DFS/RFS was assessed in 15 publications, and DSS was assessed in three publications. Most of the studies evaluated clinicopathological parameters. Among the 35 publications, 27 included patients in Asia while eight involved Caucasian patients. HR estimation was provided by the authors in 29 publications; HR values for the remaining six publications were calculated by survival curves. Most of the publications evaluated Skp2 expression by IHC or tissue array. RT-PCR and Western blot assays were carried out by Takanami [35] and Min [25].

Quality assessment

The quality assessment of the 35 publications was carried out by NOS quality scale, and 66% (23/35) scored highly (with seven stars or more). Quality scores can be found in Table S1.

Skp2 expression and OS in cancer patients

Thirty-six studies including 5,789 samples in 23 publications were eligible for the evaluation of the association between Skp2 expression and cancer OS. Results showed that Skp2 expression was significantly associated with poor OS (HR=1.702; 95% CI=1.475-1.964). In subgroup analysis, overexpression of Skp2 was also shown to be associated with poor OS in both the Asian and Caucasian population (Asian: HR=2.173, 95% CI=1.704-2.773; Caucasian: HR=1.094, 95% CI=1.005-1.191). When stratified by cancer type, studies of esophageal squamous cell carcinoma, oral squamous cell carcinoma, and head and neck squamous cell carcinoma were classified as “squamous cell carcinoma”. Studies of colorectal tumors were classified as “gastrointestinal cancer”, as were studies of gastric carcinoma. In the remaining studies, cancers with only one study for analysis were classified as “other”. Skp2 expression was found to be associated with poor OS in all cancer subgroups except ovarian cancer, gastrointestinal cancer, and NSCLC (NPC: HR=3.248, 95% CI=1.183-8.914; STS: HR=1.579, 95% CI=1.111-2.243; breast carcinoma: HR=2.256, 95% CI=1.492-3.410; squa-

Table 1. Characteristics of literatures included in the meta-analysis

Reference	Ethnicity	Cancer type	Sample size	Method	Antibody (dilution)	Cut off value	No. of positive/high	Skp2 location	Out come
Yang, 2015	Asian	Breast Carcinomas	102	IHC	Cell signaling technology (1:200)	10% (high)	High/low: 56/46	Nuclear/cytoplasmic	DFS
Wang, 2014	Asian	Nasopharyngeal carcinoma	95	IHC	Invitrogen (1:200)	131.25 (OS), 128.82 (PFS)	--	Nuclear	OS, PFS
Tian, 2013	Asian	Rectal cancer	172	IHC	Zymed (1:200)	0 positive 50% high	82 (high)	Nuclear	CF, LRFS, MEFS, DSS
Lv, 2013	Asian	Gastrointestinal Stromal Tumors	114	IHC	Zymed (1:200)	10% high	27	Nuclear	RFS
Sorbye, 2013	Caucasian	Soft tissue sarcomas	193	IHC	Zymed (1:10)	0 positive	176	Both	OS
Liu, 2013	Asian	Breast carcinoma	98	IHC	Santa (1:100)	26% high	40	Both	CP, OS
Liu, 2012	Asian	Breast carcinoma	251	IHC	Santa (1:100)	75% high	165	Cytoplasma	OS, DFS
Liang, 2012	Asian	Esophageal squamous cell carcinoma	157	TMA	Invitrogen (1:40)	0 positive	68	Nuclear	OS
Lu, 2012	Asian	Ovarian cancer	46	IHC	Zymed (1:100)	14.61% high	22	Nuclear	OS
Shin, 2012	Asian	Hepatocellular carcinoma	359	TMA	Biocare Medica (1:100)	0 positive	41, 195	Nuclear/cytoplasma	DFS, OS
Xu, 2011	Caucasian	Nasopharyngeal carcinoma	127	TMA	Invitrogen (1:40)	0 positive	96	Nuclear	OS, DFS
Chen, 2011	Caucasian	Melanoma	436	TMA	Santa (1:100)	8 high	206	Cytoplasmic/nuclear	OS, DSS
		Primary melanoma	290			124		Cytoplasmic/nuclear	
		Metastatic melanoma	146			82		Cytoplasmic/nuclear	
Nguyen, 2011	Caucasian	Prostate cancer	109	IHC	Generated by M. Pagano (1:100)	4% high	12	Nuclear	RFS
Seki, 2010	Asian (CHOP-like therapy) R-CHOP group	Diffuse large B-cell lymphoma	425	IHC	Santa	40% high	166, 91	Nuclear	OS, PFS
Lu, 2009	Asian	Hepatocellular carcinoma	74	IHC	Zymed (1:100)	24.16% high	28	Nuclear	OS
Hashimoto, 2009	Asian	Intrahepatic Cholangiocarcinomas	74	IHC	Santa (1:50)	20% high	36	Nuclear	OS
Fang, 2009	Asian	Nasopharyngeal carcinoma	233	IHC	Zymed (1:100)	85	218	Nuclear	DMF, OS
Huang, 2008	Asian	Myxofibrosarcoma	75	TMA	Zymed (1:100)	10% high	36	Nuclear	OS, MEFS
Carracedo, 2008	Caucasian	Head and neck squamous cell carcinoma	62	IHC	Zymed (1:100)	37 high	24	Nuclear	OS
Liu, 2008	Asian	Renal cell carcinoma	482	TMA	Zymed (1:100)	0 positive	71	Nuclear	CSS, RFS
Huang, 2006	Asian	Myxofibrosarcoma	70	TMA	Zymed (1:100)	10% high	34	Nuclear	OS, MEFS, DSS
Harada, 2005	Asian	Oral squamous cell carcinoma	102	IHC	Santa (1:100)	20% high	37	Nuclear	OS
Saigusa, 2005	Asian	Glioblastomas	35	IHC	Zymed (1:200)	10% high	11	Nuclear	OS
Shapira, 2005	Asian	Colorectal tumors	80	IHC	Zymed (1:100)	50% high	32	Nuclear	OS
Takanami, 2005	Asian	NSCLC	79	RT-PCR		0.38 high	46	Both	OS
Honjo, 2005	Asian	Gastric carcinoma	63	IHC	Zymed (1:50)	20% high	33	Nuclear	OS
Zhu, 2004	Caucasian	NSCLC	95	TMA	Zymed (1:100)	5 high	41	Nuclear	OS, DFS
Min, 2004	Asian	Acute Myelogenous Leukemia	99	WB	zymed	0.7 high	57	Both	DFS, OS
Sanada, 2004	Asian	Biliary tract cancers	33	IHC	Zymed (1:200)	10% high, 0 positive	18, 15	Nuclear	OS, DFS

Li, 2004	Asian	Colorectal tumors	102	IHC	Santa (1:200)	7.8% high	51	Nuclear	OS
Oliveira, 2003	Caucasian	Soft tissue sarcomas	182	IHC	Santa (1:100)	10% high	68	Nuclear	LRFS, DFS, OS, MEFS
Shigemasa, 2003	Asian	Ovarian cancer	91	IHC	Santa (1:50)	5% positive, 25% high	43 positive, 18 high	Nuclear	OS (diffuse)
Shintani, 2003	Asian	Oral squamous cell carcinomas	75	IHC	Zymed (1:100)	10% positive	45	Nuclear	OS
Dong, 2003	Asian	Laryngeal squamous cell carcinomas	102	IHC	Santa (1:50)	25% high	37	Nuclear	OS, DFS
Yang, 2002	Caucasian	Prostate cancer	622	TMA	M. Pagano (1:100)	0 positive, 10 high	557 positive, 394 high	Nuclear	RFS

Abbreviations: OS, overall survival; DFS, disease free survival; PFS, progression free survival; CF, clinicopathological features; LRFS, local recurrence free survival; MEFS, metastasis free survival; DSS, disease specific survival; RFS, relapse-free survival; CP, clinicopathological parameters; DMF, distant metastasis-free; CSS, cancer-specific survival.

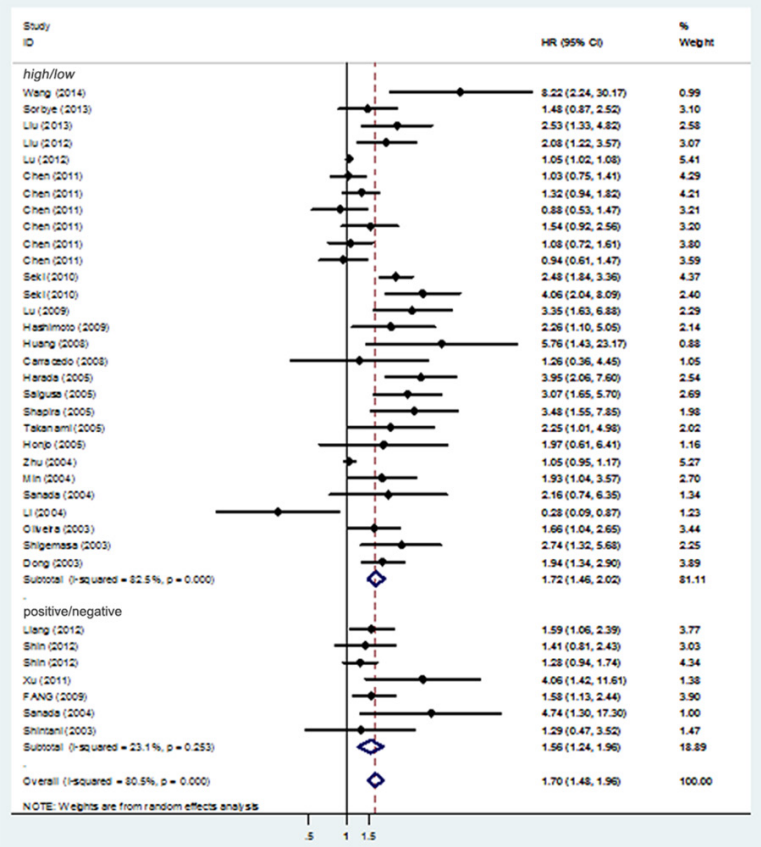

Figure 2. Forest plot of effect of Skp2 expression on cancer overall survival by subgroup analysis of "Skp2 positive/negative" and "Skp2 high/low".

mous cell carcinoma: HR=1.915, 95% CI=1.500-2.446; HCC: HR=1.697, 95% CI=1.016-2.775; gastrointestinal cancer: HR=1.282, 95% CI=0.285-5.774; NSCLC: HR=1.381, 95% CI=0.674-2.829; other cancer: HR=1.726, 95% CI=1.309-2.276). When stratified by detection method or antibody, Skp2 expression was associated with poor OS in both the IHC group and the TMA group (IHC: HR=2.146, 95% CI=1.623-2.838; TMA: HR=1.221, 95% CI=1.047-1.422). Moreover, Skp2 expression was associated with poor OS regardless of whether the antibody supplier was Invitrogen, Zymed, or Santa Cruz (Invitrogen: HR=3.256, 95% CI=1.195-8.871; Zymed: HR=1.607, 95% CI=1.317-1.960; Santa Cruz: HR=1.642, 95% CI=1.283-2.101).

The cellular localization of Skp2 might affect the association between Skp2 expression and cancer OS. In the subgroup analysis by Skp2 localization, Skp2 expression was found to be associated with poor OS when nuclear and when both nuclear and cytoplasmic (nuclear: HR=1.845, 95% CI=1.546-2.202; both: HR=1.920, 95% CI=1.402-2.628), while no association was found when localization was cytoplasmic (HR=1.173, 95% CI=0.988-1.392). In the studies included, some stratified the samples into "positive" and "negative", while others adopted different cutoff values to stratify the samples into "high" and "low". Therefore, we stratified the studies into "Skp2 positive/negative" and "Skp2 high/low" according to the different cutoff values and stratification methods used in all the studies. Since the publications included in this study utilized different cut-off values to distinguish patients, we stratified the studies into two groups: "Skp2 positive/negative" or "Skp2 high/low". Our results demonstrated significant associations in both groups (positive/negative: HR=1.513, 95% CI=1.258-1.821; high/low: HR=1.718, 95% CI=1.462-2.019) (**Figure 2**), implying that different cut-off values might not affect the association between Skp2 expression and OS.

Skp2 expression and OS/DSS in cancer patients

Four studies including 1,114 samples evaluated Skp2 levels and DSS in cancer patients. In total, 40 studies including 6,903 samples were eligible for evaluating the association between Skp2 expression and cancer OS/DSS.

Our results suggested that Skp2 expression is significantly associated with poor OS/DSS (HR=1.666, 95% CI=1.457-1.904) (**Figure 3**). In subgroup analysis, the results were in accordance with those of subgroup analysis in OS. Overexpression of Skp2 was also shown to be associated with poor prognosis in both the Asian and Caucasian population (Asian: HR=2.209, 95% CI=1.740-2.805; Caucasian: HR=1.099, 95% CI=1.015-1.190). When strati-

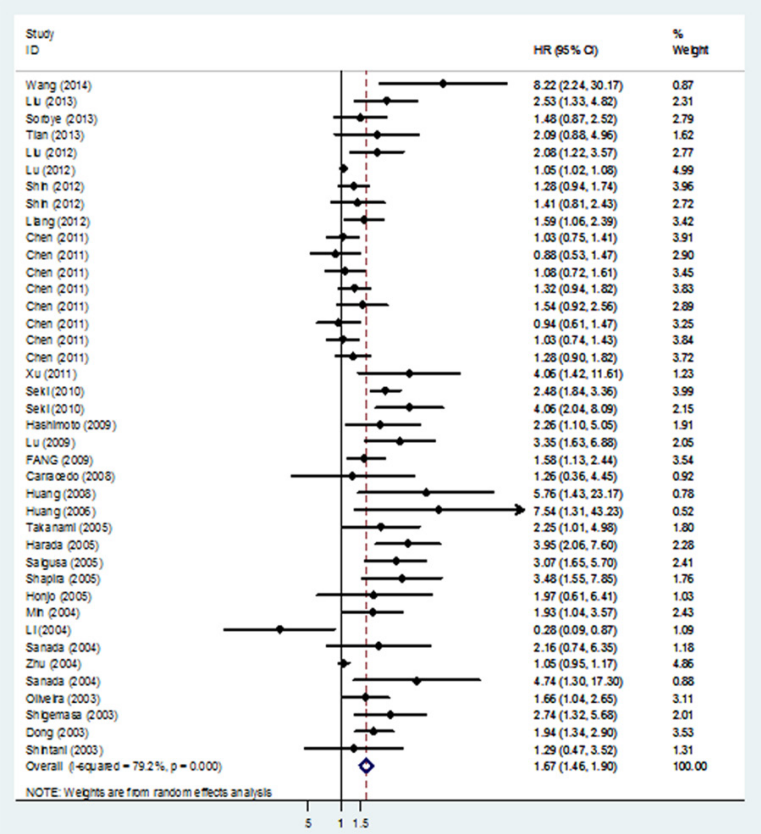

Figure 3. Forest plot of effect of Skp2 expression on cancer OS/DSS.

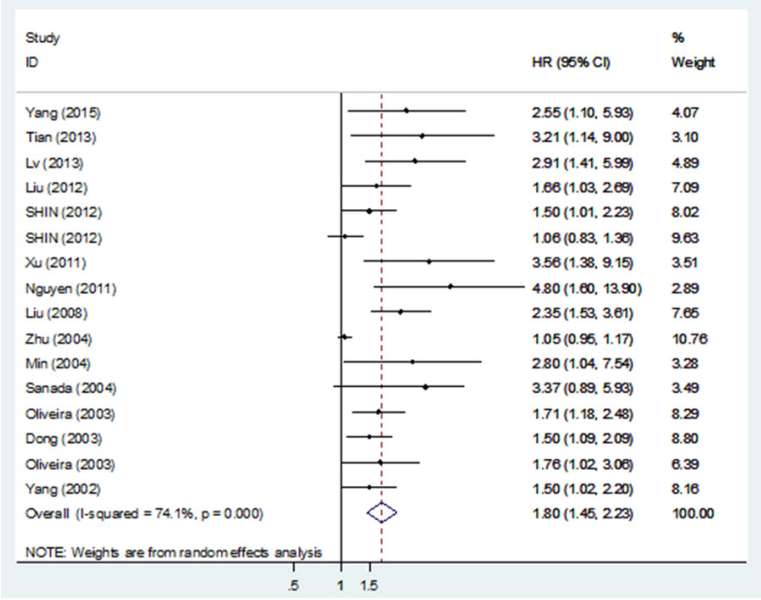

Figure 4. Forest plot of effect of Skp2 expression on cancer DFS/RFS.

fied by detection method or antibody, Skp2 expression was found to be associated with poor prognosis when assessed by IHC or TMA (IHC: HR=2.143, 95% CI=1.632-2.815; TMA: HR=1.220, 95% CI=1.062-1.401) regardless of whether the antibody supplier was Invitrogen, Zymed, or Santa Cruz (Invitrogen: HR=3.256, 95% CI=1.195-8.871; Zymed: HR=1.677, 95% CI=1.374-2.048; Santa Cruz: HR=1.3563, 95% CI= 1.256-1.946).

Subgroup analysis by Skp2 localization also showed that the cellular localization of Skp2 might affect the association between Skp2 expression and cancer prognosis. Skp2 expression was found to be associated with poor OS/DSS when nuclear and when both nuclear and cytoplasmic (nuclear: HR=1.835, 95% CI= 1.551-2.171; both: HR=1.920, 95% CI=1.402-2.628), while no association was found when localization was cytoplasmic only (HR=1.148, 95% CI= 0.952-1.383). Significant association between Skp2 expression and cancer OS/DSS was also found in both groups: "Skp2 positive/negative" and "Skp2 high/low expression" (positive/negative: HR= 1.561, 95% CI=1.241-1.963; high/low: HR=1.673, 95% CI= 1.442-1.941). The subgroup analysis results showed the consistency of the relationship between Skp2 expression and OS and DSS.

Skp2 expression and DFS/RFS in cancer patients

Studies reporting either DFS or RFS were also included to explore the association between Skp2 expression and cancer DFS/RFS. There were 16 studies including 3,390 eligible patients, among which six

Table 2. Meta-analysis of effects of Skp2 expression on OS and DFS/DSS/RFS

	n[a]	Sample size	HR (95% CI)	P[b]	I^2 (P^c)
Total					
OS	36	5789	1.702 (1.475-1.964)[R]	**0.000**	80.5% (0.000)
DFS/DSS/RFS	20	4504	1.701 (1.414-2.04[5])[R]	**0.000**	70.6% (0.000)
Ethniticity					
Asian					
OS	26	3513	2.173 (1.704-2.773)[R]	**0.000**	85.4% (0.000)
DFS/DSS/RFS	13	2442	2.002 (1.547-2.58[9])R	**0.000**	59.1% (0.004)
Caucasian					
OS	10	2276	1.094 (1.005-1.191)	**0.038**	2.1% (0.419)
DFS/DSS/RFS	7	2062	1.372 (1.088-1.731)[R]	**0.008**	68.3% (0.004)
Cancer type (OS)					
NPC	3	455	3.248 (1.183-8.914)[R]	**0.022**	74.1% (0.021)
STS	2	375	1.579 (1.111-2.243)	**0.011**	0.00% (0.751)
Breast carcinoma	2	349	2.256 (1.492-3.410)	**0.000**	0.00% (0.652)
Ovarian cancer	2	137	1.576 (0.622-3.989)[R]	0.337	84.9% (0.010)
Squamous cell carcinoma	5	498	1.915 (1.500-2.446)	**0.000**	38.9% (0.162)
HCC	3	792	1.697 (1.016-2.775)[R]	**0.043**	65.9% (0.053)
Gastrointestinal cancer	3	245	1.282 (0.285-5.774)[R]	0.746	84.3% (0.002)
NSCLC	2	174	1.381 (0.674-2.829)[R]	0.377	71.0% (0.063)
Other	14	2764	1.726 (1.309-2.276)[R]	**0.000**	73.8% (0.000)
Method					
IHC					
OS	22	2695	2.146 (1.623-2.838)[R]	**0.000**	86.2% (0.000)
DFS/DSS/RFS	10	1419	1.902 (1.572-2.300)	**0.000**	7.6% (0.372)
TMA					
OS	12	2916	1.221 (1.047-1.422)[R]	**0.011**	47.7% (0.033)
DFS/DSS/RFS	9	2986	1.382 (1.114-1.715)[R]	**0.003**	71.7% (0.000)
Antibdoy					
Invitrogen					
OS	3	379	3.256 (1.195-8.871)[R]		73.3% (0.024)
DFS/DSS/RFS	2	241	3.135 (1.766-5.567)	**0.000**	0.0% (0.740)
Zymed					
OS	14	1196	1.607 (1.317-1.960)[R]		75.4% (0.000)
DFS/DSS/RFS	7	1123	2.337 (1.355-4.028)[R]	**0.002**	81.1% (0.000)
Santa cruz					
OS	16	3417	1.642 (1.283-2.101)[R]		75.6% (0.000)
DFS/DSS/RFS	6	1589	1.410 (1.187-1.675)	**0.000**	17.0% (0.304)
Skp2 location					
Nuclear					
OS	27	3838	1.845 (1.546-2.202)[R]	**0.000**	83.6% (0.000)
DFS/DSS/RFS	15	3257	1.867 (1.474-2.364)[R]	**0.000**	74.5% (0.000)
Cytoplasmic					
OS	5	1482	1.173 (0.988-1.392)	0.069	40.6% (0.151)
DFS/DSS/RFS	3	1046	1.145 (0.904-1.451)	0.262	33.8% (0.221)
Both					
OS	4	469	1.920 (1.402-2.628)	**0.000**	0.0% (0.621)
DFS/DSS/RFS	2	201	2.652 (1.395-5.041)	**0.003**	0.0% (0.890)

Positive or high					
Positive/negative					
OS	7	1343	1.513 (1.258-1.821)	**0.000**	23.1% (0.253)
DFS/DSS/RFS	4	1327	1.716 (1.074-2.742)[R]	**0.024**	79.1% (0.002)
High/low					
OS	29	4446	1.718 (1.462-2.019)[R]	**0.000**	82.5% (0.000)
DFS/DSS/RFS	16	3177	1.723 (1.390-2.135)[R]	**0.000**	69.4% (0.000)
Outcome (DFS/DSS/RFS)					
RFS	6	1681	2.153 (1.604-2.889)	**0.000**	30.8% (0.204)
DSS	4	1114	1.397 (0.915-2.132)[R]	0.122	55.4% (0.081)
DFS	10	1709	1.560 (1.239-1.964)[R]	**0.000**	70.9% (0.000)

[a]Number of comparisons. [b]*P* value of Z-test for pooled OR. The OR values with statistical significance were shown in bold ($p<0.05$). [c]*P* value of Q-test for heterogeneity test. [R]Random-effects model was used when *P* value for heterogeneity test < 0.05; otherwise, fixed-effects model was used. Abbreviations: OS, overall survival; DSS, disease specific survival; DFS, disease free survival; RFS, relapse-free survival.

studies reported cancer RFS and ten reported cancer DFS.

Overall, high Skp2 expression was significantly associated with poor DFS/RFS (HR=1.798, 95% CI=1.451-2.227) (**Figure 4**). The subgroup analysis was carried out by population, detection method, detection antibody, Skp2 distribution, cut-off value, and outcome. Skp2 expression was associated with poor DFS/RFS in both the Asian (HR=1.937, 95% CI=1.486-2.524) and Caucasian (HR=1.575, 95% CI=1.098-2.260) population.

Detection method and commercial antibody source did not affect the analysis results, as Skp2 expression was associated with poor DFS/RFS both when assessed by IHC and when assessed by TMA (IHC: HR=1.933, 95% CI=1.564-2.388; TMA: HR=1.451, 95% CI=1.101-1.912), and regardless of whether the antibody was purchased from Invitrogen, Zymed, or Santa Cruz (Invitrogen: HR=3.135, 95% CI=1.766-5.567; Zymed: HR=2.156, 95% CI=1.173-3.966; Santa Cruz: HR=1.623, 95% CI=1.325-1.988). The localization of Skp2 affected the association between Skp2 expression and DFS/RFS. When stratified by Skp2 localization, Skp2 expression was associated with poor DFS/RFS when nuclear (HR=1.901, 95% CI=1.456-2.484) but not when cytoplasmic (HR=1.263, 95% CI=0.822-1.942). Significant association between Skp2 expression and cancer DFS was also found in both groups: "Skp2 positive/negative" and "Skp2 high/low expression" (positive/negative: HR=1.716, 95% CI=1.074-2.742; high/low: HR=1.878, 95% CI=1.437-2.454). When stratified by outcome, Skp2 expression was correlated significantly with cancer RFS and DFS (RFS: HR=2.135, 95% CI=1.604-2.889; DFS: HR=1.560, 95% CI=1.239-1.964).

Heterogeneity and sensitivity evaluation

There was heterogeneity among studies in overall and subgroup analyses (**Table 2**); therefore, the random-effect model was used in most of the analyses. To evaluate the sensitivity of our meta-analysis, we sequentially removed each individual study from the pooled HR. The results demonstrated that our meta-analysis was statistically reliable (**Figure 5**).

Potential publication bias

Publication bias in our meta-analysis was evaluated by funnel plots and Egger's tests. As shown in **Figure 6**, the shape of the funnel plot was symmetrical indicating no evidence of publication bias in our analysis.

Discussion

Skp2 has been reported to be overexpressed in various cancers since its discovery as the specific substrate-targeting subunit of SCF [40]. As an F-box protein of SCF, Skp2 also mediates the degradation of many tumor suppressors by ubiquitylation, such as p21, p57, p103, E-cadherin, and RhoE. Skp2 promotes p21 degradation to regulate cancer cell senescence, and also degrades E-cadherin to promote EMT. Moreover, RhoE protein, which is involved in the regulation of cancer cell proliferation, survival,

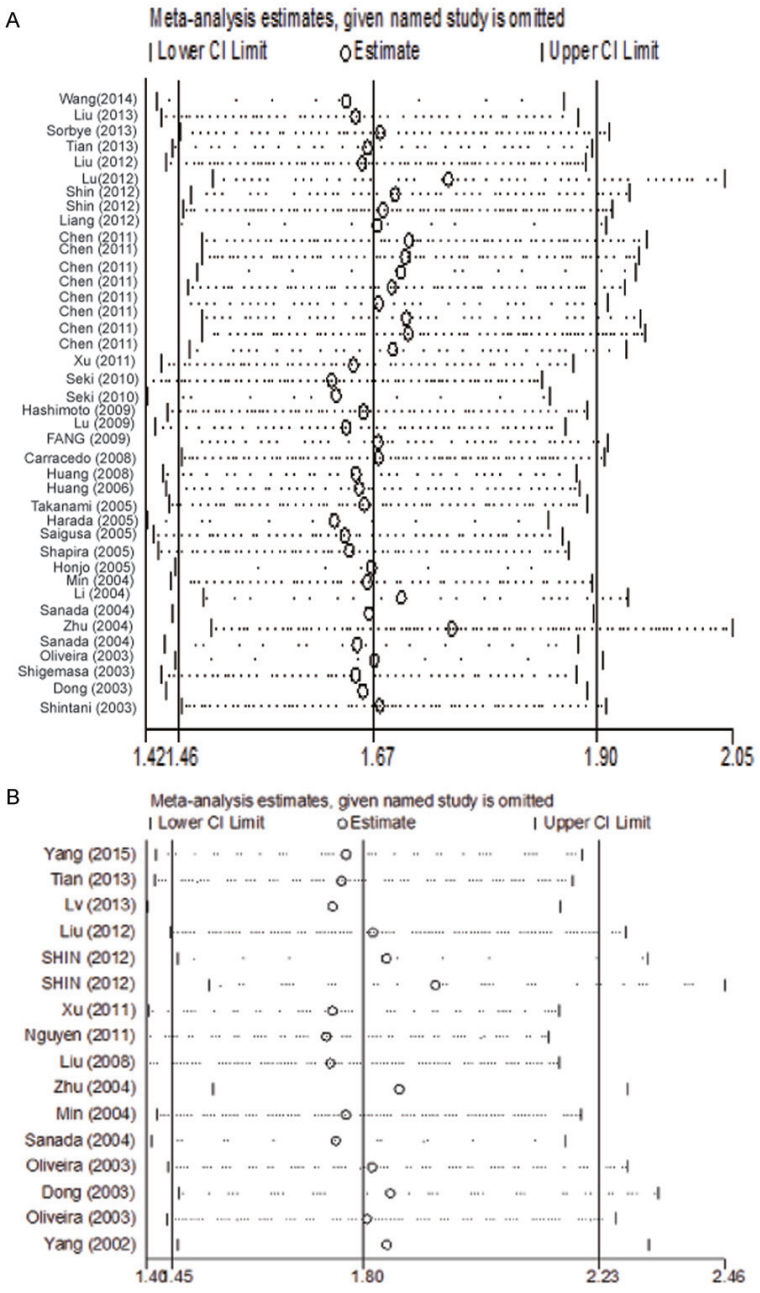

Figure 5. Sensitivity analysis of the impact of Skp2 expression on cancer OS/DSS (A) and DFS/RFS (B).

and metastasis, is also degraded by the proteasome via Skp2. Skp2 also has SCF-independent functions, such as disruption of the Rb and p53 pathways by binding to the transcriptional coactivator p300 [41-43].

Thus, it is believed that multiple signaling pathways, such as phosphatidylinositol 3-kinase (PI3K)/Akt, p27, PTEN, and AR, cross-talk with Skp2 in various cancers to promote tumor progression [44-46]. Besides accelerating cell growth by degrading p27, the overexpression of Skp2 was also reported to be associated with tumor cell migration, invasion, metastasis, EMT, poor tumor differentiation, and poor cancer prognosis. Thus, it was speculated that Skp2 may be associated with tumor prognosis, and studies have been carried out to explore the association between skp2 expression and cancer survival.

In this meta-analysis, we explored the prognostic value of Skp2 expression in cancer. Our results suggested that high Skp2 expression is associated with poor OS and DFS/RFS in cancer patients. The prognostic impact of Skp2 expression on OS, OS/DSS, and DFS/RFS remained significant in subgroup analyses including the analysis of population, analysis method, detection antibody, cancer type, nuclear Skp2, outcome, and cut-off values, but excluding the analysis of cytoplasmic Skp2, ovarian cancer, gastrointestinal cancer, NSCLC, and M. Pagano (the subgroup in which the antibody from "M. Pagano" was used).

The lack of association between Skp2 and cancer prognosis in some subgroups may be due to the limited number of studies in those groups. In the future, more studies should be included to validate our results. Another reason may be Skp2 isoforms. Cytoplasmic Skp2 is an alternative splice form, Skp2B, characterized by the presence of a unique C-terminal domain. Cytoplasmic Skp2B does not regulate p27 levels [47-49]. Therefore, the staining of Skp2 in the cytoplasm might interfere with experiment results and may have consequently affected our analysis results. Skp2 cellular localization should be taken into

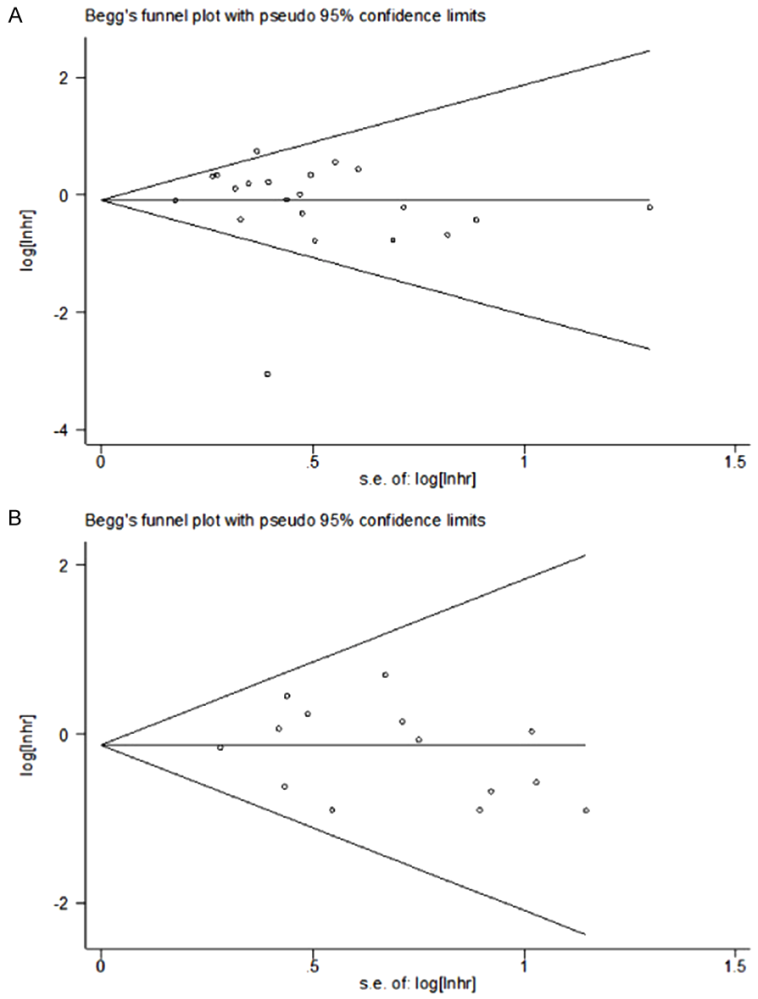

Figure 6. Funnel plots for the evaluation of potential publication bias in the impact of Skp2 expression on OS/DSS (A) and DFS/RFS (B).

consideration when evaluating the value of Skp2 expression in the assessment of cancer prognosis.

Many groups reported that high expression of Skp2 is associated with the poor survival of cancer patients. To the best of our knowledge, this meta-analysis is the first study to systematically assess the association between Skp2 expression and prognosis in various cancers, and the data are consistent with the known function of Skp2 in disease. One limitation of our meta-analysis is the heterogeneity between studies. This heterogeneity may originate from inconsistent parameters in evaluation procedures, such as cut-off values, experimental methods, and patient populations. For example, of the 35 publications included in this analysis, 26% (9/35) scored only one star in the exposure section, indicating that the overall quality for exposure was comparatively low and that heterogeneity existed between studies. However, our sensitivity analysis demonstrated that the results were statically stable, and that the heterogeneity did not affect the analysis results. In the future, standard and normalized parameters should be used when assessing Skp2 expression and cancer prognosis, which may be helpful for the evaluation of the clinical impact of Skp2 levels.

Conclusion

Our analysis revealed a significant association between Skp2 expression and OS, OS/DSS, and DFS/RFS in various cancers; therefore, Skp2 might be a predictive and independent marker of prognosis. However, since our analysis has a few limitations, the results should be interpreted with caution. Large prospective clinical studies based on a rigorously designed methodology and homogeneous cohorts of patients are needed to further confirm the prognostic value of Skp2 in different types of cancer.

Acknowledgements

This work was supported by grant for Medical and health science and technology plan of Zhejiang province (grant No.2015KYB090), the Major Science and Technology Innovation Project of Hangzhou (20112313A01), the National Natural Science Foundation (81172072), the Science Foundation for Distinguished Young Scholars of Zhejiang (R2101405), China.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiaoju Wang, Center for Molecular Medicine, Zhejiang Academy of Medical Sciences, Hangzhou, 310013 Zhejiang, P.R.

China. E-mail: wang.x.george@gmail.com; Dr. Xiaoming Sun, Department of Forensic Medicine, Xuzhou Medical College, Xuzhou, 221002 Jiangsu, P.R. China. E-mail: lupusstar@163.com

References

[1] Wang G, Chan CH, Gao Y and Lin HK. Novel roles of Skp2 E3 ligase in cellular senescence, cancer progression, and metastasis. Chin J Cancer 2012; 31: 169-177.

[2] Yan L, Yun N, Xiu-Min D and Xu-Qi X. Oncogenic role of Skp2 and p27Kip1 in intraductal proliferative lesions of the breast. Chin Med Sci J 2012; 27: 161-166.

[3] Egozi D, Shapira M, Paor G, Ben-Izhak O, Skorecki K and Hershko DD. Regulation of the cell cycle inhibitor p27 and its ubiquitin ligase Skp2 in differentiation of human embryonic stem cells. FASEB J 2007; 21: 2807-2817.

[4] Yang G, Ayala G, De Marzo A, Tian W, Frolov A, Wheeler TM, Thompson TC and Harper JW. Elevated Skp2 protein expression in human prostate cancer: association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. Clin Cancer Res 2002; 8: 3419-3426.

[5] Honjo S, Kase S, Osaki M, Ardyanto TD, Kaibara N and Ito H. COX-2 correlates with F-box protein, Skp2 expression and prognosis in human gastric carcinoma. Int J Oncol 2005; 26: 353-360.

[6] Traub F, Mengel M, Luck HJ, Kreipe HH and von Wasielewski R. Prognostic impact of Skp2 and p27 in human breast cancer. Breast Cancer Res Treat 2006; 99: 185-191.

[7] Lu M, Ma J, Xue W, Cheng C, Wang Y, Zhao Y, Ke Q, Liu H, Liu Y, Li P, Cui X, He S and Shen A. The expression and prognosis of FOXO3a and Skp2 in human hepatocellular carcinoma. Pathol Oncol Res 2009; 15: 679-687.

[8] Seki R, Ohshima K, Fujisaki T, Uike N, Kawano F, Gondo H, Makino S, Eto T, Moriuchi Y, Taguchi F, Kamimura T, Tsuda H, Shimoda K and Okamura T. Prognostic significance of S-phase kinase-associated protein 2 and p27kip1 in patients with diffuse large B-cell lymphoma: effects of rituximab. Ann Oncol 2010; 21: 833-841.

[9] Guo ZQ, Lu QS, Zhang YP, Wang Z, Tang ZY, Zhang Y, Wang W, Yang HJ, Lu N, Wen XW, Chen CP and Wu WX. [Expression of SKP2 and Thr187 phosphorylated p27(kip1) proteins in human breast carcinoma and their prognostic significance]. Zhonghua Bing Li Xue Za Zhi 2013; 42: 197-198.

[10] Lv A, Li Z, Tian X, Guan X, Zhao M, Dong B and Hao C. SKP2 high expression, KIT exon 11 deletions, and gastrointestinal bleeding as predictors of poor prognosis in primary gastrointestinal stromal tumors. PLoS One 2013; 8: e62951.

[11] Carracedo DG, Astudillo A, Rodrigo JP, Suarez C and Gonzalez MV. Skp2, p27kip1 and EGFR assessment in head and neck squamous cell carcinoma: prognostic implications. Oncol Rep 2008; 20: 589-595.

[12] Chen G, Cheng Y, Zhang Z, Martinka M and Li G. Cytoplasmic Skp2 expression is increased in human melanoma and correlated with patient survival. PLoS One 2011; 6: e17578.

[13] Dong Y, Sui L, Watanabe Y, Sugimoto K and Tokuda M. S-phase kinase-associated protein 2 expression in laryngeal squamous cell carcinomas and its prognostic implications. Oncol Rep 2003; 10: 321-325.

[14] Fang FM, Chien CY, Li CF, Shiu WY, Chen CH and Huang HY. Effect of S-phase kinase-associated protein 2 expression on distant metastasis and survival in nasopharyngeal carcinoma patients. Int J Radiat Oncol Biol Phys 2009; 73: 202-207.

[15] Harada K, Supriatno, Kawaguchi S, Kawashima Y, Itashiki Y, Yoshida H and Sato M. High expression of S-phase kinase-associated protein 2 (Skp2) is a strong prognostic marker in oral squamous cell carcinoma patients treated by UFT in combination with radiation. Anticancer Res 2005; 25: 2471-2475.

[16] Hashimoto N, Yachida S, Okano K, Wakabayashi H, Imaida K, Kurokohchi K, Masaki T, Kinoshita H, Tominaga M, Ajiki T, Ku Y, Okabayashi T, Hanazaki K, Hiroi M, Izumi S, Mano S, Okada S, Karasawa Y, Maeba T and Suzuki Y. Immunohistochemically detected expression of p27(Kip1) and Skp2 predicts survival in patients with intrahepatic cholangiocarcinomas. Ann Surg Oncol 2009; 16: 395-403.

[17] Huang HY, Huang WW, Wu JM, Huang CK, Wang JW, Eng HL, Lin CN, Chou SC, Yu SC, Fang FM, Lee JC and Li CF. Flow cytometric analysis of DNA ploidy and S-phase fraction in primary localized myxofibrosarcoma: correlations with clinicopathological factors, Skp2 expression, and patient survival. Ann Surg Oncol 2008; 15: 2239-2249.

[18] Huang HY, Kang HY, Li CF, Eng HL, Chou SC, Lin CN and Hsiung CY. Skp2 overexpression is highly representative of intrinsic biological aggressiveness and independently associated with poor prognosis in primary localized myxofibrosarcomas. Clin Cancer Res 2006; 12: 487-498.

[19] Li JQ, Wu F, Masaki T, Kubo A, Fujita J, Dixon DA, Beauchamp RD, Ishida T, Kuriyama S and Imaida K. Correlation of Skp2 with carcinogenesis, invasion, metastasis, and prognosis in colorectal tumors. Int J Oncol 2004; 25: 87-95.

[20] Liang Y, Hou X, Cui Q, Kang TB, Fu JH, Zhang LJ, Luo RZ, He JH, Zeng YX and Yang HX. Skp2 expression unfavorably impacts survival in resectable esophageal squamous cell carcinoma. J Transl Med 2012; 10: 73.
[21] Liu J, Wei XL, Huang WH, Chen CF, Bai JW and Zhang GJ. Cytoplasmic Skp2 expression is associated with p-Akt1 and predicts poor prognosis in human breast carcinomas. PLoS One 2012; 7: e52675.
[22] Liu X, Wang H, Ma J, Xu J, Sheng C, Yang S, Sun L and Ni Q. The expression and prognosis of Emi1 and Skp2 in breast carcinoma: associated with PI3K/Akt pathway and cell proliferation. Med Oncol 2013; 30: 735.
[23] Liu Z, Fu Q, Lv J, Wang F and Ding K. Prognostic implication of p27Kip1, Skp2 and Cks1 expression in renal cell carcinoma: a tissue microarray study. J Exp Clin Cancer Res 2008; 27: 51.
[24] Lu M, Zhao Y, Xu F, Wang Y, Xiang J and Chen D. The expression and prognosis of FOXO3a and Skp2 in human ovarian cancer. Med Oncol 2012; 29: 3409-3415.
[25] Min YH, Cheong JW, Lee MH, Kim JY, Lee ST, Hahn JS and Ko YW. Elevated S-phase kinase-associated protein 2 protein expression in acute myelogenous leukemia: its association with constitutive phosphorylation of phosphatase and tensin homologue protein and poor prognosis. Clin Cancer Res 2004; 10: 5123-5130.
[26] Nguyen PL, Lin DI, Lei J, Fiorentino M, Mueller E, Weinstein MH, Pagano M and Loda M. The impact of Skp2 overexpression on recurrence-free survival following radical prostatectomy. Urol Oncol 2011; 29: 302-308.
[27] Oliveira AM, Okuno SH, Nascimento AG and Lloyd RV. Skp2 protein expression in soft tissue sarcomas. J Clin Oncol 2003; 21: 722-727.
[28] Saigusa K, Hashimoto N, Tsuda H, Yokoi S, Maruno M, Yoshimine T, Aoyagi M, Ohno K, Imoto I and Inazawa J. Overexpressed Skp2 within 5p amplification detected by array-based comparative genomic hybridization is associated with poor prognosis of glioblastomas. Cancer Sci 2005; 96: 676-683.
[29] Sanada T, Yokoi S, Arii S, Yasui K, Imoto I and Inazawa J. Skp2 overexpression is a p27Kip1-independent predictor of poor prognosis in patients with biliary tract cancers. Cancer Sci 2004; 95: 969-976.
[30] Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I and Hershko DD. The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. Cancer 2005; 103: 1336-1346.
[31] Shigemasa K, Gu L, O'Brien TJ and Ohama K. Skp2 overexpression is a prognostic factor in patients with ovarian adenocarcinoma. Clin Cancer Res 2003; 9: 1756-1763.
[32] Shin E, Kim SH, Jeong HY, Jang JJ and Lee K. Nuclear expression of S-phase kinase-associated protein 2 predicts poor prognosis of hepatocellular carcinoma. APMIS 2012; 120: 349-357.
[33] Shintani S, Li C, Mihara M, Hino S, Nakashiro K and Hamakawa H. Skp2 and Jab1 expression are associated with inverse expression of p27(KIP1) and poor prognosis in oral squamous cell carcinomas. Oncology 2003; 65: 355-362.
[34] Sorbye SW, Kilvaer TK, Valkov A, Donnem T, Smeland E, Al-Shibli K, Bremnes RM and Busund LT. Prognostic impact of Jab1, p16, p21, p62, Ki67 and Skp2 in soft tissue sarcomas. PLoS One 2012; 7: e47068.
[35] Takanami I. The prognostic value of overexpression of Skp2 mRNA in non-small cell lung cancer. Oncol Rep 2005; 13: 727-731.
[36] Tian YF, Chen TJ, Lin CY, Chen LT, Lin LC, Hsing CH, Lee SW, Sheu MJ, Lee HH, Shiue YL, Huang HY, Pan HY, Li CF and Chen SH. SKP2 overexpression is associated with a poor prognosis of rectal cancer treated with chemoradiotherapy and represents a therapeutic target with high potential. Tumour Biol 2013; 34: 1107-1117.
[37] Wang J, Huang Y, Guan Z, Zhang JL, Su HK, Zhang W, Yue CF, Guan S and Liu QQ. E3-ligase Skp2 predicts poor prognosis and maintains cancer stem cell pool in nasopharyngeal carcinoma. Oncotarget 2014; 5: 5591-601.
[38] Xu HM, Liang Y, Chen Q, Wu QN, Guo YM, Shen GP, Zhang RH, He ZW, Zeng YX, Xie FY and Kang TB. Correlation of Skp2 overexpression to prognosis of patients with nasopharyngeal carcinoma from South China. Chin J Cancer 2011; 30: 204-212.
[39] Zhu CQ, Blackhall FH, Pintilie M, Iyengar P, Liu N, Ho J, Chomiak T, Lau D, Winton T, Shepherd FA and Tsao MS. Skp2 gene copy number aberrations are common in non-small cell lung carcinoma, and its overexpression in tumors with ras mutation is a poor prognostic marker. Clin Cancer Res 2004; 10: 1984-1991.
[40] Qi M, Liu D, Zhang S, Hu P and Sang T. Inhibition of S-phase kinase-associated protein 2-mediated p27 degradation suppresses tumorigenesis and the progression of hepatocellular carcinoma. Mol Med Rep 2015; 11: 3934-3940.
[41] Inuzuka H, Gao D, Finley LW, Yang W, Wan L, Fukushima H, Chin YR, Zhai B, Shaik S, Lau AW, Wang Z, Gygi SP, Nakayama K, Teruya-Feldstein J, Toker A, Haigis MC, Pandolfi PP and Wei W. Acetylation-dependent regulation of Skp2 function. Cell 2012; 150: 179-193.
[42] Lonjedo M, Poch E, Mocholi E, Hernandez-Sanchez M, Ivorra C, Franke TF, Guasch RM and

Perez-Roger I. The Rho family member RhoE interacts with Skp2 and is degraded at the proteasome during cell cycle progression. J Biol Chem 2013; 288: 30872-30882.

[43] Yang Q, Huang J, Wu Q, Cai Y, Zhu L, Lu X, Chen S, Chen C and Wang Z. Acquisition of epithelial-mesenchymal transition is associated with Skp2 expression in paclitaxel-resistant breast cancer cells. Br J Cancer 2014; 110: 1958-1967.

[44] Bochis OV, Irimie A, Pichler M and Berindan-Neagoe I. The role of Skp2 and its substrate CDKN1B (p27) in colorectal cancer. J Gastrointestin Liver Dis 2015; 24: 225-234.

[45] Li B, Lu W, Yang Q, Yu X, Matusik RJ and Chen Z. Skp2 regulates androgen receptor through ubiquitin-mediated degradation independent of Akt/mTOR pathways in prostate cancer. Prostate 2014; 74: 421-432.

[46] Li B, Lu W and Chen Z. Regulation of androgen receptor by E3 ubiquitin ligases: for more or less. Receptors Clin Investig 2014; 1.

[47] Drobnjak M, Melamed J, Taneja S, Melzer K, Wieczorek R, Levinson B, Zeleniuch-Jacquotte A, Polsky D, Ferrara J, Perez-Soler R, Cordon-Cardo C, Pagano M and Osman I. Altered expression of p27 and Skp2 proteins in prostate cancer of African-American patients. Clin Cancer Res 2003; 9: 2613-2619.

[48] Germain D. Skp2 and Skp2B team up against Rb and p53. Cell Div 2011; 6: 1.

[49] Radke S, Pirkmaier A and Germain D. Differential expression of the F-box proteins Skp2 and Skp2B in breast cancer. Oncogene 2005; 24: 3448-3458.

Table S1. Assessment of publication quality included in the meta-analysis

Reference	Selection	Comparability	Exposure	Total scores
Yang (2015)	☆☆☆	☆☆	☆☆☆	8
Wang (2014)	☆☆☆	☆☆	☆	6
Tian (2013)	☆☆☆	☆☆	☆☆☆☆	9
Lv (2013)	☆☆☆	☆	☆☆☆	7
Sorbye (2013)	☆☆☆	☆☆	☆☆☆	8
Liu (2013)	☆☆☆	☆☆	☆☆☆	8
Liu (2012)	☆☆☆	☆☆	☆	6
Liang (2012)	☆☆☆	☆☆	☆☆☆	8
Lu (2012)	☆☆☆	☆☆	☆	6
Shin (2012)	☆☆☆	☆☆	☆☆☆	8
Xu (2011)	☆☆	☆☆	☆	5
Chen (2011)	☆☆	☆☆	☆☆☆	7
Nguyen (2011)	☆☆☆	☆☆	☆☆	7
Seki (2010)	☆☆☆	☆☆	☆	6
Lu (2009)	☆☆☆	☆☆	☆☆☆	8
Hashimoto (2009)	☆☆☆	☆☆	☆☆☆	8
Fang (2009)	☆☆☆	☆☆	☆☆	7
Huang (2008)	☆☆☆	☆☆	☆☆☆	8
Carracedo (2008)	☆☆☆	☆☆	☆☆	7
Liu (2008)	☆☆	☆☆	☆☆	6
Huang (2006)	☆☆☆	☆☆	☆☆☆	8
Harada (2005)	☆☆☆	☆☆	☆	6
Saigusa (2005)	☆☆	☆	☆☆	5
Shapira (2005)	☆☆☆	☆☆	☆	6
Takanami (2005)	☆☆☆	☆☆	☆☆☆	8
Honjo (2005)	☆☆	☆☆	☆☆	6
Zhu (2004)	☆☆☆	☆☆	☆☆	7
Min (2004)	☆☆	☆☆	☆	5
Sanada (2004)	☆	☆☆	☆	4
Li (2004)	☆☆☆	☆☆	☆☆☆	8
Oliveira (2003)	☆☆☆	☆☆	☆☆	7
Shigemasa (2003)	☆☆☆	☆☆	☆☆	7
Shintani (2003)	☆☆☆	☆☆	☆☆	7
Dong (2003)	☆☆☆	☆☆	☆☆☆	8
Yang (2002)	☆☆☆	☆☆	☆☆☆	8