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Original Article

Prognostic roles of PCNA expressions in non-small cell lung cancer: a meta-analysis

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Abstract: Background: Proliferating cell nuclear antigen (PCNA) may act as a prognostic biomarker in human cancers. Objective: This study is prepared to clarify the prognostic value of PCNA in patients with non-small cell lung cancer (NSCLC). Methods: All eligible articles from MEDLINE, EMBASE, CENTRAL, and Chinese BioMedical Literature Database were incorporated into this study. We extracted the patients' clinical characteristics and survival outcomes and performed a meta-analysis to demonstrate the prognostic role of PCNA and the correlations between PCNA expression and clinical characteristics. Results: Eighteen studies with a total of 2716 patients were included in this meta-analysis. PCNA expression level of SCC or miscellaneous cancers was significantly higher than ADs (χ^2=3.99, P=0.046; χ^2=4.39, P=0.036) and there is no significant difference of PCNA expressions between SCC and miscellaneous cancers (χ^2=1.25, P=0.264). PCNA expression could predict the poor prognosis of patients with NSCLC. The combined hazard ratio (HR, 95% CI) was 1.35 (1.12, 1.62) for overall survival (OS) and 1.71 (0.87, 3.34) for progression free survival or disease free survival. The combined HR (95% CI) of OS was 1.10 (0.57, 2.13) for ADs and 0.97 (0.65, 1.45) for Stage I NSCLC. The PCNA expression was associated with primary tumor stage with OR=2.93 (95% CI 1.54-5.57), lymph node metastasis status with OR=1.65 (95% CI 1.04-2.63), tumor node metastasis stage with OR=1.81 (95% CI 1.28-2.56) and vascular invasion with OR=0.40 (95% CI 0.17-0.93). Conclusions: PCNA might play an unfavorable prognostic role for overall survival in NSCLC patients. Further rigorous and high-quality investigations on the effectiveness of PCNA as a therapeutic target for NSCLC are warranted.

Keywords: Non-small cell lung cancers, PCNA, prognosis, meta-analysis

Introduction

Lung cancer is the leading cause of cancer-related death around the world with about 1.4 million deaths worldwide each year [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 80 percent of all lung cancer cases. Although some advances have been made in treatments, lung cancer has an extremely poor prognosis, with a five-year overall survival of 16% in the USA and less than 10% in the UK [2]. Alone or in combination with other factors, the prognostic factors are variable measured indicators in individual patients which may explain parts of the population heterogeneity, and are able to provide information on clinical outcomes at the time of diagnosis. The tumor-node-metastasis (TNM) staging system has been thought to have an effect on survival in NSCLC patients, however, some problems such as similar prognosis for patients with different tumor stages and various prognosis for patients with the same tumor stage have been indicated. At the same time, recent researches have reported that some biological markers may have an impact on survival in NSCLC patients [3-5].

The dynamic equilibrium between proliferation and apoptosis is vital for a tissue in controlling its growth and these factors may, therefore, also prove valuable to evaluate a patient's prognosis. Proliferating cell nuclear antigen (PCNA), also known as cyclin or auxiliary protein for DNA polymerase δ [6] and DNA polymerase ε [7], is a highly conserved 36 kDa acid nuclear protein, which is associated with promoting cell proliferation [6] and maintaining the fidelity of mammalian DNA replication [29]. Expression of PCNA rises at the end of G1 period, reaches its peak in S-phase, drops during G2 phase, and disappears during the mitotic phase and in qui-

#1 lung carcinoma* [Title/Abstract]
#2 lung cancer* [Title/Abstract]
#3 lung tumo* [Title/Abstract]
#4 lung neoplasms [MeSH Terms]
#5 #1 OR #2 OR #3 OR #4
#6 PCNA [Title/Abstract]
#7 proliferating cell nuclear antigen [Title/Abstract]
#8 #6 OR #7
#9 prognos* [Title/Abstract]
#10 #5 AND #8 AND #9

Figure 1. Search strategy of PubMed.

escent cells [6, 8]. PCNA was first discovered with the use of autoantibodies present in some systemic lupus erythematosus (SLE) patients [9]. Nuclear immunoreactivity of PCNA was both found in the proliferative compartment of normal tissues and tumors, so PCNA was deemed as a marker of cell proliferation. Some studies have indicated that PCNA was correlated with survival in patients with malignant tumors, including breast cancer [10], rectal cancer [11], hepatocellular carcinoma [12], renal cell carcinoma [13], as well as NSCLC [20, 21]. However, the results concerning the prognostic role of PCNA expression in NSCLC are inconsistent among clinical studies. Thus, it is still unknown whether PCNA could serve as a prognostic biomarker in NSCLC.

The aim of our study is to discover the prognostic value of PCNA expression in NSCLC. At the same time, we investigate the correlation between PCNA expression and clinical characteristics.

Materials and methods

This systematic review and meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement protocol [14].

Search strategy

We searched MEDLINE (via PubMed), EMBASE (via OVID), CENTRAL (via Cochrane Library), and Chinese BioMedical Literature Database (CBM) to Feb 2015 to identify studies relevant to this review. Our search strategy included the following subject headings and/or key words variably combined by “lung neoplasm”, “PCNA”, and “prognosis”. The detailed search strategy of PubMed is shown in **Figure 1**. In addition, reference lists of the articles initially detected were searched by hand to identify additional relevant reports. The eligibility of references retrieved by the search was assessed independently by two of the authors (Jun Fan and Xudong Zhou), and the review authors resolved differences of opinion by discussion or by appeal to a third review author (Jian Huang) when necessary. The full text of the remaining articles, including the references, was examined to determine whether the articles contained relevant information.

Inclusion and exclusion criteria

Studies were considered eligible if they met all of the following inclusion criteria (1) study population consisting of primary NSCLC patients; (2) PCNA expression was evaluated in primary lung carcinoma tissues; (3) the association between PCNA expression and overall survival (OS), disease free survival (DFS) were measured and/or the associations of PCNA expression and clinical characteristics was reported. Studies were excluded based on any of the following criteria: (1) the following article types were excluded: reviews, letters, laboratory researches, animal experiments; (2) the language is not English or Chinese; (3) it lacked critical data for hazard ratio (HR) analysis.

Quality assessment

Quality assessment of individual studies was performed independently by two of the authors (Jun Fan and Xudong Zhou), using the Newcastle-Ottawa Scale for cohort studies. The scale allocates stars (maximum of 9) for quality of selection, comparability and outcome of study participants [15]. Any discrepancies were addressed by joint reevaluation of the original article.

Data extraction

Data were extracted from the selected studies independently by two of the authors (Jun Fan and Xudong Zhou), using a predefined standardized form and resolved disagreements by discussion between two review authors or by appeal to a third review author (Xin Wang). The original data including the expression numbers of PCNA, the Kaplan-Meier (K-M) survival curves or HR and 95% confidence interval (CI) of survival outcomes. Multivariate Cox hazard regression analysis data is our priority, but if not obtained, univariate Cox hazard regression analysis or K-M survival curves with log-rank *p* value of survival outcomes were instead. In our meta-analysis, since published HR was not

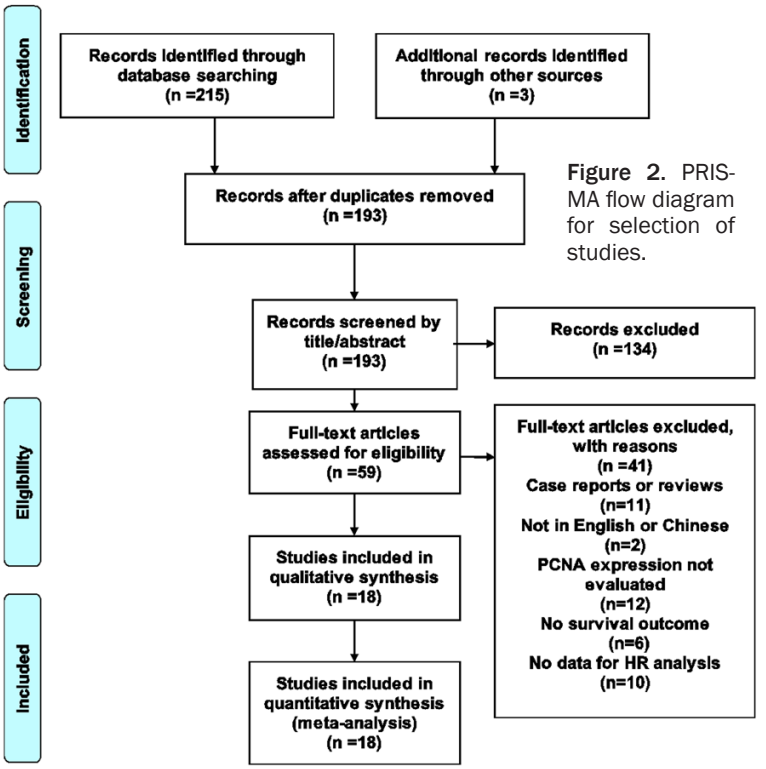

Figure 2. PRISMA flow diagram for selection of studies.

available in included studies, we calculated the HR with 95% CI using survival rates, enrolled samples and corresponding *P* value from log-rank test in accordance with the described instructions. The relevant formulas are given as follows:

$$O - E = \frac{\sqrt{Total\ observed\ events \times Analyzed\ research \times Analyzed\ control}}{(Analyzed\ research + Analyzed\ control)} \times (Z\ score\ for\ P\ value/2)$$

$$V = \frac{Total\ observed\ events \times Analyzed\ research \times Analyzed\ control}{(Analyzed\ research + Analyzed\ control)}$$

$$HR = \text{Exp}\left(-\frac{O - E}{V}\right)$$

where O-E is the log rank Observed minus Expected events and V is the log rank Variance [16]. Then we extracted the associated details by Engauge Digitizer 4.1 (http://sourceforge.net) from the K-M curves to measure the accuracy of estimated HR. Moreover, we extracted data of the eligible articles' basic characteristics, including first author (year), country, study period, type of study, No. of patients, No. of patients evaluated PCNA expression and/or survival data, median follow-up, patients' mean age when diagnosed lung cancer, tumor type, cut-off value, and attitude conclusion.

Statistical analysis

The log hazard ratio was chosen as the appropriate summary statistics because it was the only summary statistic that allows for both censoring and time to an event [17]. But these relevant statistical variables were not given explicitly in most studies, and instead we extracted associated data from the K-M survival curves. We carried out meta-analysis on PCNA expression in NSCLC cells for OS and DFS. We also analyzed correlations between PCNA expression and clinical characteristics includingage, gender, differentiation, smoking history, primary tumor (pT) stage, tumor-node-metastasis (TNM) stage, lymph node metastasis, and other characteristics. According to clinical characteristics, Stage-I and Stage-II, Stage-III and Stage-IV, T1 and T2, and T3 and T4 were combined; Well differentiation (G1) and moderate differentiation (G2) were combined and poor differentiation (G3) was separated. Their correlations were described by odds ratio (OR). The effects of PCNA expression on survival outcome (OS/DFS) and correlations between PCNA expression and clinical characteristics were estimated by forest plots. Heterogeneity was defined as $P<0.10$ or $I^2>70\%$. When homogeneity was fine ($P>0.10$, $I^2<70\%$), a fixed effect model was used to combine effective sizes. On the contrary, a random effect model was used. Subgroup analyses were performed to investigate the potential causes of heterogeneity according to regions, sample size, follow-up period and NOS scores. In addition, Meta-regression was alsoused to identify the source of heterogeneity. An observed HR>1 indicated a worse outcome for the positive group compared to the negative group and was considered significant if the 95% CI did not overlap 1. The potential publication bias was evaluated by Begg's rank correlation and Egger's test, with $P>0.05$ indicating no potential publication bias [18]. Meta-analysis and publication biases were both performed by STATA13.0 (STATA Corporation, College Station, TX). Additionally, the difference of PCNA expressions in different

Table 1. Baseline characteristics of included studies

Study	Country	Language	Study period	Type of study	N	n	Age (years)	Follow-up (months)	Cut-off value	Dilution	Method	Antibody	Survival outcome	Attitude
Yonechi, 2014 [19]	Japan	English	2004-2005	retrospective	54	38	69.7	17	50% stain	1/3000	IHC	Monoclonal, mouse anti-human PCNA, Abcam	OS	negative
Wang, 2014 [20]	China	English	2005-2008	retrospective	180	180	NR	60	10% stain	NR	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS,DFS	positive
Chen, 2013 [21]	China	English	2000-2006	retrospective	42	42	69	64	10% stain	1/400	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	negative
Oka, 2011 [22]	Japan	English	2003-2007	retrospective	296	183	70	53.7	50% stain	1/500	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS,DFS	negative
Liu, 2011 [23]	China	English	2000-2005	retrospective	452	452	56	NR	30% stain	1/50	IHC	Monoclonal, mouse anti-human PCNA, Kyoto	OS	negative
Dworakowska, 2009 [24]	Poland	English	1998-2005	retrospective	170	122	60	60	NR	NR	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS,DFS	negative
Weng, 2008 [25]	China	Chinese	2000-2003	retrospective	86	86	56	NR	50% stain	NR	IHC	Monoclonal, mouse anti-human PCNA, BOSTER	OS	negative
Grossi, 2003 [26]	Italy	English	1990-1996	retrospective	269	239	64	NR	10% stain	1/200	IHC	Monoclonal, mouse anti-human PCNA, BioGenex	OS	negative
Dworakowska, 2002 [27]	Poland	English	1994	retrospective	95	95	59.4	74	10% stain	1/200	IHC	Monoclonal, mouse anti-human PCNA, DAKO	OS	negative
Volm and Koomagi, 2000 [28]	Germany	English	NR	retrospective	150	142	58	NR	NR	1/10	IHC	Monoclonal, mouse anti-human PCNA, Dianova	OS	positive
Nguyen, 2000 [29]	Czech	English	NR	retrospective	89	49	NR	NR	50% stain	NR	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	negative
Fukuse, 2000 [30]	Japan	English	1988-1993	retrospective	242	242	62.3	NR	20% stain	1/500	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	negative
Demarchi, 2000 [31]	Brazil	English	1979-1994	retrospective	64	64	59.8	51.9	45.16% stain	1/80	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	negative
Fukuse, 1999 [32]	Japan	English	1979-1993	retrospective	34	34	64.3	64	20% stain	1/500	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	positive
Hirata, 1998 [33]	Japan	English	1981-1990	retrospective	69	69	62.6	76	20% stain	NR	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	negative
Ebina, 1994 [34]	USA	English	1984-1990	retrospective	123	123	55.3	NR	50% stain	NR	IHC	Monoclonal, mouse anti-human PCNA, Dako	OS	positive
Ishida, 1993 [35]	Japan	English	1974-1987	retrospective	211	211	63	NR	5% stain	1/100	IHC	Monoclonal, mouse anti-human PCNA, Coulter	OS	positive
Fujii, 1993 [36]	Japan	English	1983-1990	retrospective	90	87	63	NR	50% stain	1/25	IHC	Monoclonal, mouse anti-human PCNA, Novocastra	OS	positive

N: No. of patients included in the study; n: No. of testing PCNA and analyzing survival; NR: not refer; OS: overall survival; DFS: disease-free survival; IHC: immunohistochemistry; PCNA: proliferating cell nuclear antigen.

Table 2. Quality assessment of individual studies using the Newcastle-Ottawa Scale (NOS) for cohort studies

Study	Selection												Comparability		Outcome								Score
	a				b			c					d		e		f				g		h				
	a1*	a2*	a3	a4	b1*	b2	b3	c1*	c2*	c3	c4	d1*	d2	e1*	e2*	f1*	f2*	f3	f4	g1*	g2	h1*	h2*	h3	h4	
Yonechi, 2014 [19]		*			*			*						*			*						*			6
Wang, 2014 [20]	*				*			*						*			*			*		*				7
Chen, 2013 [21]	*				*			*						*			*			*		*				7
Oka, 2011 [22]	*				*			*						*			*						*			6
Liu, 2011 [23]	*				*			*						*			*					*				6
Dworakowska, 2009 [24]	*				*			*						*			*			*			*			7
Weng, 2008 [25]	*				*			*						*			*					*				6
Grossi, 2003 [26]		*			*			*						*			*						*			6
Dworakowska, 2002 [27]	*				*			*						*			*			*		*				7
Volm and Koomagi, 2000 [28]		*			*			*						*			*						*			6
Nguyen, 2000 [29]	*				*			*						*			*						*			6
Fukuse, 2000 [30]	*				*			*						*			*					*				6
Demarchi, 2000 [31]	*				*			*						*			*					*				6
Fukuse, 1999 [32]	*				*			*						*			*			*		*				7
Hirata, 1998 [33]	*				*			*						*			*			*		*				7
Ebina, 1994 [34]	*				*			*						*			*					*				6
Ishida, 1993 [35]	*				*			*						*			*					*				6
Fujii, 1993 [36]	*				*			*						*			*						*			6

a: Representativeness of the exposed cohort; a1: truly representative; a2: somewhat representative; a3: selected group of users; a4: no description of the derivation of the cohort; b: Selection of the non-exposed cohort; b1: drawn from the same community as the exposed cohort; b2: drawn from a different source; b3: no description of the derivation of the non-exposed cohort; c: Ascertainment of exposure; c1: secure record; c2: structured interview; c3: written self-report; c4: no description; d: Demonstration that outcome of interest was not present at start of study; d1: yes; d2: no; e: Comparability of cohorts on the basis of the design or analysis; e1: study controls for the most important factor; e2: study controls for any additional factor; f: Assessment of outcome; f1: independent blind assessment; f2: record linkage; f3: self-report; f4: no description; g: Was follow-up long enough for outcomes to occur; g1: yes; g2: no; h: Adequacy of follow up of cohorts; h1: complete follow up-all subjects accounted for; h2: subjects lost to follow up unlikely to introduce bias-small number lost >80% follow up, or description provided of those lost; h3: follow up rate <80% and no description of those lost; h4: no statement.

Table 3. Chi square test of expression of PCNA in different pathological types of NSCLC

Tissue Type	PCNA Positive	PCNA Negative	Total	Chi-square value	*P* value
ADs	437	403	840	7.37	0.025
SCC	202	144	346		
Miscellaneous Cancers	39	20	59		
Total	678	567	1245		

ADs: adenocarcinomas, SCC: squamous cell carcinoma, PCNA: proliferating cell nuclear antigen.

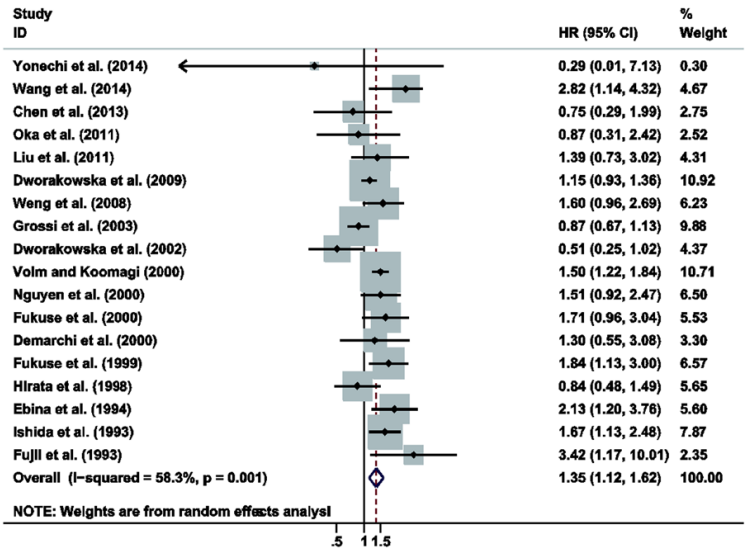

Figure 3. Evaluated hazard ratios summary for the expression of PCNA in NSCLC for overall survival.

pathological types of NSCLC was evaluated by Chi square test on SPSS 19 (SPSS Inc., Chicago), with $P<0.05$ indicating significant.

Results

Reference retrieval

After primary retrieval, a total of 218 potentially relevant studies were incorporated into our initial study, including 85 in Medline, 57 in Embase, 70 in CBM, 3 in CENTRAL and 3 by reference list. 25 were excluded for duplicates and 134 were excluded by title/abstract screening. Full texts were retrieved for the remaining 59 studies. Finally 18 retrospective trials [19-36] met all the criteria for inclusion in the analysis (**Figure 2**), which included 2716 patients with a median number of 150.9 patients per study.

Characteristics and qualities of the included studies

The clinical characteristics of patients and other useful information are listed in **Table 1**. 13 studies were published after 2000, and the other 5 studies were published in the 1990s. Non-small cell lung cancer trials included either all histological subtypes [19-21, 23-30, 32-36] (n=16), or adenocarcinoma [22, 31] (n=2) or squamous cell cancer (n=0). Data related to local advanced disease (stages I-III) comprised ten [19, 20, 22, 25-28, 30, 31, 35] of the 18 NSCLC trials. Three [21, 23, 33] of the 18 NSCLC studies were performed in local early disease (stages I), while five [24, 29, 32, 34, 36] were dealt with any stage (stages I-IV). Immunohistochemistry techniques (IHC) were used in all the trials to detect the expression of PCNA protein. Various antibodies were used to assess PCNA expression, however, all of the studies used the same clone (PC 10 clone).

Quality assessments of individual studies were shown in **Table 2**. We used the Newcastle-Ottawa Scale (NOS) for cohort studies to assess included studies, which included 3 aspects (selection, comparability and outcome) and 8 items. All studies scored either 6 or 7.

PCNA

Difference of PCNA expressions in different pathological types of NSCLC: 10 articles [19, 22, 23, 25, 27, 29, 31, 32, 34, 36] including 1245 objects (840 patients of adenocarcinomas (ADs) in 10 articles [19, 22, 23, 25, 27, 29, 31, 32, 34, 36], 346 patients of squamous cell carcinoma (SCC) in 8 articles [19, 23, 25, 27, 29, 32, 34, 36] and 59 patients of miscellaneous cancers in 4 articles [27, 29, 34, 36]) evaluated the difference of PCNA expression in different pathological types of NSCLC (**Table 3**). Positive expression of PCNA was found in

Table 4. Meta-analyses of PCNA expression to predict the survival outcome in NSCLC patients

Tumor type	Outcome	N	Patients	Heterogeneity (I^2, P)	Model	HR (95% CI)	*P* value	Conclusion
NSCLC	OS	18	2716	58.3%, 0.001	Random	1.35 (1.12, 1.62)	0.001	positive
	DFS	3	485	89.2%, 0.000	Random	1.71 (0.87, 3.34)	0.118	negative
ADs	OS	2	247	0.0%, 0.557	Fixed	1.10 (0.57, 2.13)	0.774	negative
Stage I	OS	3	563	0.0%, 0.475	Fixed	0.97 (0.65, 1.45)	0.882	negative

ADs: adenocarcinomas, SCC: squamous cell carcinoma, NSCLC: non-small cell lung cancer, N reference count, OS: overall survival, DFS: disease free survival, HR: hazard ratio, CI: confidence interval.

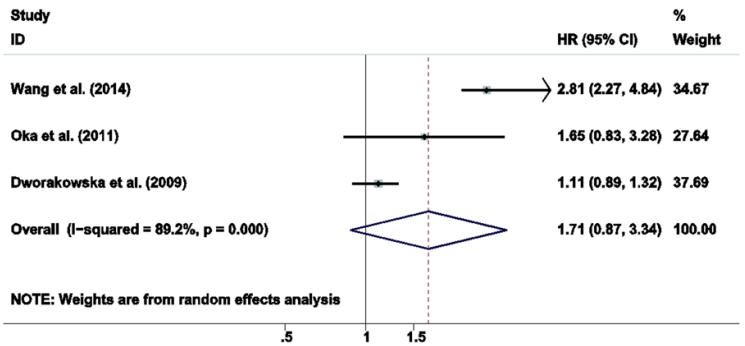

Figure 4. Evaluated hazard ratios summary for the expression of PCNA in NSCLC for disease free survival.

52.0% of ADs, 58.4% of SCC and 66.1% of miscellaneous cancers (χ^2=7.37, P=0.025). Furthermore, we examined the difference of PCNA expression between two different pathological types. As a result, PCNA expression level of SCC or miscellaneous cancers was significantly higher than ADs (χ^2=3.99, *P*=0.046; χ^2=4.39, *P*=0.036) and there is no significant difference of PCNA expressions between SCC and miscellaneous cancers (χ^2=1.25, P=0.264).

Correlation between PCNA expression and survival outcomes

All articles [19-36] including 2716 patients listed the relationship between PCNA expression of NSCLC and survival outcomes. The combined HR (95% CI) was 1.35 (1.12, 1.62) (I^2=58.3%, P=0.001) (**Figure 3**; **Table 4**) for OS in all these studies, but was 1.71 (0.87, 3.34) (n=485, I^2=89.2%, P=0.000) (**Figure 4**; **Table 4**) for DFS in three studies [20, 22, 24]. Positive expression of PCNA was found to be a predictor of poor prognosis for OS (not for DFS) in NSCLC patients. Subgroup analyses were performed according to histology and extent of the disease. Two trials for ADs were assessable (**Table 4**); the combined HR (95% CI) was 1.10 (0.57, 2.13) (I^2=0.0%, *P*=0.557). When we aggregated the 3 studies that reported results for Stage I NSCLC, the combined HR was 0.97 (0.65, 1.45) (I^2=0.0%, *P*=0.475). So positive expression of PCNA maybe not a predictor of poor prognosis for OS in Stage I NSCLC or ADs patients. Moderate heterogeneity was found in the meta-analysis for HR (OS) of the prognostic role of PCNA expression. Univariable meta-regression was used to identify the source of heterogeneity, however, the publish year (*P*=0.366), cut-off of staining (*P*=0.290), average age (*P*=0.209) and number of patients (*P*=0.869) could not explain the heterogeneity.We also conducted a subgroup analysis according to regions, sample size, follow-up period and NOS scores (**Table 6**) and none of them could explain the heterogeneity.

Correlation between PCNA expression and clinical characteristics

The studies that referred to the correlation between PCNA expression and clinical characteristics were gathered to evaluate the combined ORs. We found that positive PCNA expression was significantly correlated with pT stage with OR=2.93 (95% CI 1.54-5.57), N status with OR=1.65 (95% CI 1.04-2.63), TNM stage with OR=1.81 (95% CI 1.28-2.56) and vascular invasion with OR=0.40 (95% CI 0.17-0.93). All the results including OR (95% CI) and *P* value are listed in **Table 5**.

Assessment of publication bias

The publication bias is a major concern for all forms of meta-analyses, because positive results tend to be accepted by journals while negative results are often be rejected or even not be submitted. Two methods including Begg's funnel plot and Egger's test were used to eva-

Table 5. Meta-analyses of PCNA expression classified by patient characteristics

Clinical characteristics	N	Patients	Heterogeneity (I-squared, P)	Model	OR (95% CI)	*P* value	Conclusion
pT stage (T3-T4 vs. T1-T2)	4	281	0.0%, 0.825	Fixed	2.93 (1.54, 5.57)	0.001	positive
Age (>60 vs. ≤60)	4	371	0.0%, 0.745	Fixed	0.99 (0.64, 1.54)	0.956	negative
Gender (male vs. female)	8	1307	21.5%, 0.259	Fixed	1.13 (0.88, 1.47)	0.344	negative
Smoking history (smoker vs. non-smoker)	2	635	0.0%, 0.683	Fixed	0.89 (0.63, 1.24)	0.479	negative
Differentiation (G3 vs. G1-G2)	5	739	87.9%, 0.000	Random	1.74 (0.40, 7.58)	0.462	negative
TNM Stage (III-IV vs. I-II)	6	666	0.9%, 0.411	Fixed	1.81 (1.28, 2.56)	0.001	positive
N status (N1-N3 vs. N0)	5	461	0.0%, 0.506	Fixed	1.65 (1.04, 2.63)	0.035	positive
Recurrence (yes vs. no)	1	183	--	--	1.06 (0.37, 3.02)	0.915	negative
Vascular invasion (yea vs. no)	1	90	--	--	0.40 (0.17, 0.93)	0.033	positive
ECOG performance status (0 vs. 1-2)	1	452	--	--	1.27 (0.72, 2.21)	0.409	negative
Serum CEA level (>2.5 vs. ≤2.5)	1	180	--	--	1.23 (0.53, 2.85)	0.635	negative
Visceral pleural involvement (yea vs. no)	1	452	--	--	1.49 (0.97, 2.31)	0.071	negative
Lobe location (right vs. left)	1	452	--	--	1.19 (0.81, 1.74)	0.381	negative

pT stage: primary tumor stage, TNM stage: tumor node metastasis stage, N reference count, N status lymph node metastasis status, ECOG: Eastern Cooperative Oncology Group, CEA: carcinoembryonic antigen, OR: odds ratio, CI: confidence interval.

Table 6. Subgroup analyses of the relationships between PCNA expression and overall survival (OS)

Comparison Variables	Number of studies (I^2 statistics %)	HR (95% CI), *P* value	Heterogeneity between sub-groups (*P* value)
Total	18 (58.3%)	1.35 (1.12-1.62), 0.000	NA
Regions			0.460
Asian Countries	12 (43.9%)	1.34 (1.7-1.53), 0.000	
Western Countries	6 (75.7%)	1.24 (1.08-1.43), 0.003	
Sample size			0.392
>100	8 (51.7%)	1.17 (0.91-1.50), 0.215	
≤100	10 (64.7%)	1.32 (1.18-1.46), 0.000	
Follow-up period			0.097
Referred	9 (58.9%)	1.38 (1.22-1.57), 0.046	
No referred	9 (56.8%)	1.17 (1.00-1.36), 0.000	
NOS scores			0.139
≤6	12 (47.8%)	1.37 (1.21-1.55), 0.000	
>6	6 (71.4%)	1.18 (1.01-1.38), 0.043	

ICH: immunohistochemistry, NOS: Newcastle-Ottawa Scale, HR: hazard ratio.

luate publication bias of the meta-analysis. No publication bias of the prognostic value of PCNA for OS in NSCLC was discovered on Begg's funnel plot (**Figure 5**), and the Egger's *P* value was 0.670. Though little publication bias was detected in our study, we have to warn that a poor sensitivity of Begg's funnel plot and Egger's test when the number of eligible articles is less than 20.

Discussion

To our knowledge, it is the first time that a comprehensive and detailed meta-analysis was performed to evaluate the prognostic role of PCNA in NSCLC. We discussed the difference of PCNA expressions in different pathological types of NSCLC, as well as the prognostic role of PCNA expression in NSCLC. And PCNA expression had some correlations with different clinical characteristics.

PCNA, which was regarded as a marker of cell proliferation, expressed higher in human lung cancers than in noncancerous lung tissues. In the current study, we found PCNA expression level of SCC or miscellaneous cancers was significantly higher than ADs, but not found differ-

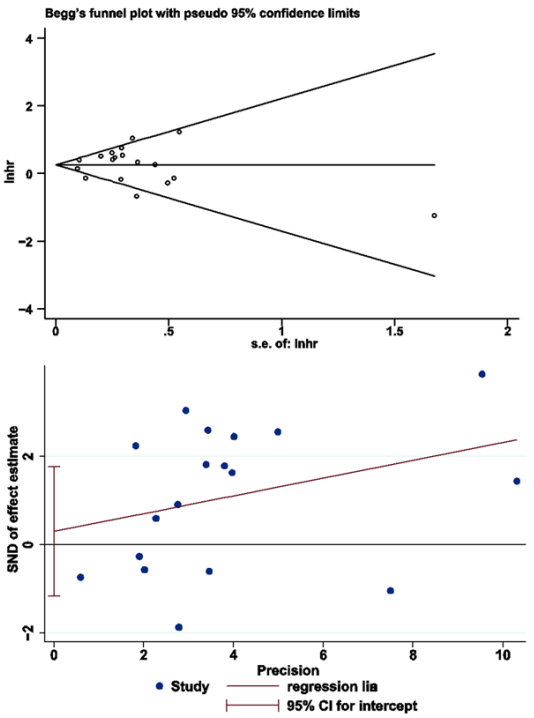

Figure 5. Publication bias of the prognostic value of PCNA for overall survival in non-small cell lung cancer on Begg's funnel plot and Egger's test.

ence between SCC and miscellaneous cancers. PCNA in SCC was significantly higher than that in ADs may be explained by the properties of these two pathological types, since SCC commonly was a central-type lung cancer which may have more activities of cell proliferation and tend to grow bigger than ADs which, in contrast, usually was a peripheral lung cancer. However, the difference of PCNA expression between miscellaneous cancers and ADs should be further investigated in consideration of limitation of eligible articles associated with miscellaneous cancers.

In the present meta-analysis, we have combined 18 published studies including 2,716 patients with NSCLC to yield summary statistics that indicate that PCNA overexpression has a significant correlation with poor overall survival in NSCLC, however, it isn't a unfavorable prognostic factor for DFS in NSCLC, which suggest that PCNA expression might be not related to recurrence, in accordance with the result of Liu [23]. When analysis was restricted to stage I NSCLC, we did not observe a statistically significant association between PCNA expression and poor overall survival. In addition, we also found that PCNA expression is not a negative prognostic factor for OS in ADs. Moreover, the associations between PCNA expression and clinical characteristics showed that the combined OR of positive PCNA expression is correlated with pT stage, N status, TNM stage and vascular invasion. These results indicated that positive expression of PCNA could be a wicked marker to predict the stage, vascular invasion and lymph node metastasis in NSCLC, which are related to survival outcome.

However, our study has several limitations. First, the findings of a meta-analysis depend on the qualities of the individual studies, as their potential problems and biases may affect the pooled effects. According to the quality assessment by NOS, twelve of the eighteen involved studies scored 6, and the other six scored 7, which indicated the qualities of all of the studies were only moderate. Second, the method of extrapolation of HR is potentially biased. If these statistics were not reported by the authors, we calculated them from the data available in the article; if this was not possible, we extrapolated them from the survival curves; so some subjective data may affect the final conclusion. Third, we didn't search unpublished and grey literature database, which may lead to a potential publication bias. Furthermore, there is also a language bias, for we only screened the literature in English and Chinese.

In conclusion, this meta-analysis implied that PCNA, which is associated with the stage, vascular invasion and lymph node metastasis, might play an unfavorable prognostic role for overall survival in NSCLC patients. However there is a moderate heterogeneity between the studies and further rigorous and high-quality investigations on the effectiveness of PCNA as a therapeutic target for NSCLC are warranted.

Disclosure of conflict of interest

None.

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