Archives of Gynecology and Obstetrics (2019) 299:1415–1427
https://doi.org/10.1007/s00404-019-05082-3

GYNECOLOGIC ONCOLOGY

High Ki-67 expression is significantly associated with poor prognosis of ovarian cancer patients: evidence from a meta-analysis

Dongmei Qiu[1] · **Wanqiu Cai**[1] · **Zhiqiao Zhang**[2] · **Hongyan Li**[1] · **Dongmei Zhou**[1]

Received: 5 April 2018 / Accepted: 2 February 2019 / Published online: 13 February 2019

Abstract

Objective The prognostic significance of Ki-67 expression in patients with ovarian cancer was controversial in various studies. Therefore, we carried out a meta-analysis to determine the prognostic significance of Ki-67 in ovarian cancer patients.
Methods We searched PubMed, Cochrane Library, EMBASE, Web of Knowledge, China National Knowledge Infrastructure database and WanFang digital database for eligible studies from January 1, 1990 to June 1, 2017. The pooled hazard ratios and 95% confidence intervals were calculated to assess the prognostic significance of Ki-67 expression for overall survival in ovarian cancer patients.
Results Finally, 38 eligible studies and 5004 ovarian cancer patients were included in the current study. The pooled hazard ratio was 1.35 (95% confidence interval 1.24–1.46, $P = 0.001$) for overall survival in ovarian cancer patients. The funnel plot bias was obviously asymmetrical and Egger's test also detected significant publication bias ($P = 0.001$). The Contour-enhanced funnel plot with trim-and-fill method supplemented 11 dummy studies to balance the funnel plot and nine new supplementary studies were in area with statistical significance. Sensitivity analysis and cumulative meta-analysis further demonstrated that the association between high Ki-67 expression and poor overall survival of ovarian cancer patients was stable and reliable.
Conclusions High Ki-67 expression is significantly related to poor overall survival and may serve as a prognostic biomarker for ovarian cancer patients.

Keywords Ovarian cancer · ki-67 · Meta-analysis · Prognostic value

Introduction

Ovarian cancer (OC) is the fifth most common cause of death induced by cancer among women with a mortality rate of approximately 54% [1]. In the United States of America, there were 22,280 newly diagnosed ovarian cancer patients and 14,240 women died of ovarian cancer in 2016 [1]. Although great progress has been made in surgery and chemotherapy, the overall cure rate remains around 30% [2]. In consideration of poor prognosis of ovarian cancer, it is necessary to identify a reliable prognostic biomarker for effectively evaluating the prognosis in OC patients.

Ki-67 is a nuclear protein which expresses throughout the cell cycle in proliferating cells, except for quiescent (G0) cells [3]. The monoclonal Ki-67 antibody MIB-1 binds to the nuclear Ki-67 antigen [4]. The relations between Ki-67 expression and prognosis of OC patients were controversial in various studies [5–42]. Several studies have found that high Ki-67 expression was correlated with poor overall

---

Dongmei Qiu and Wanqiu Cai contributed equally to this study and were co-first authors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00404-019-05082-3) contains supplementary material, which is available to authorized users.

✉ Zhiqiao Zhang
sdgrxjbk@163.com

[1] Department of Obstetrics and Gynecology, The Affiliated Chencun Hospital of Shunde Hospital of Southern Medical University, Shunde 528300, Guangdong, People's Republic of China

[2] Department of Internal Medicine, The Affiliated Chencun Hospital of Shunde Hospital, Southern Medical University, Shunde 528300, Guangdong, People's Republic of China

survival (OS) in OC patients [7, 8, 14], whereas several studies have reported that high Ki-67 expression was related with favorable OS in OC patients [9, 24, 28]. These conflicting conclusions in different studies hindered the clinical applicability of Ki-67 expression in OC patients. Therefore, we performed this meta-analysis to ascertain the prognostic significance of Ki-67 expression in OC patients.

Methods

Search strategy

The following databases, including PubMed, EMABASE, Web of Knowledge, Cochrane library, China National Knowledge Infrastructure database and WanFang digital database, were searched for eligible studies from January 1, 1990 to June 1, 2017. The search strategy combined "MeSH word" and "text word" was performed using the following words: ("MIB-1"or "Ki67" or "Ki-67" or "MIB1" or "MKI67") and ("tumor" or "neoplasm" or "carcinoma" or "malignancy" or "cancer") and ("Ovary" or "Ovarian") and ("survival" or "prognostic" or "prognosis" or "mortality" or "outcome"). The search strategy in the present study was adjusted according to the guiding principles of the relevant database. A manual literature search was carried out based on the references of relevant studies to include potential eligible studies. We further contacted the corresponding authors to get the necessary study information. There were no restrictions on the language of publication. We carried out data collection and analysis in accordance with the principles of the Helsinki Declaration.

Criteria of inclusion and exclusion

The inclusion criteria in current study were as follows: (1) patients with diagnosis of ovarian cancer by pathology; (2) Ki-67 expression was measured by immunohistochemistry method; (3) studies provided enough survival information such as hazard ratio and confidence interval. Studies without hazard ratio and confidence interval were enrolled if the survival data could be extracted from survival curves. Only the recent study was included in the current study for repeated studies from the same study cohort. There were no restrictions on race, language, sample size, or duration of follow-up.

The exclusion criteria were as follows: (1) studies without original study information, including corresponding letters, systematic review, and conference abstracts; (2) non-human experimental studies; and (3) studies lack of survival information.

Quality assessment of studies

Two independent reviewers (Zhiqiao Zhang and Dongmei Qiu) evaluated the included studies through a Newcastle–Ottawa Quality Assessment Scale (Table 1). The Newcastle–Ottawa Quality Assessment Scale (NOS) consisted of patient selection, study outcome, follow-up period, and study comparability. In course of literature assessment, the disagreements between Zhiqiao Zhang and Dongmei Qiu were settled by consensus with the third independent reviewer (Wanqiu Cai).

Data extraction

Two reviewers (Zhiqiao Zhang and Dongmei Qiu) independently extracted study data from original studies: country, the first author, sample size, publication time, assessment method for Ki-67 expression, tumor pathological stage, and survival data. All study data were noted through a standardized information table. All studies cited in the current study were indicated by the name of the first author and publishing time. The third investigator (Wanqiu Cai) helped to reach a consensus in the course of data extraction.

Statistical analyses

The statistical analyses were carried out in accordance with the principles of the Meta-analysis of Observational Studies in Epidemiology group (MOOSE) [43]. We directly recorded the original pooled HR and 95% CI from the original text. While HR and 95% CI were not provided in the text, survival information was obtained through Kaplan–Meier survival curves. The I^2 tests and Q were carried out to evaluate the clinical heterogeneity. $I^2 \geq 30\%$ and $P < 0.1$ were defined as the threshold for significant heterogeneity. A random effect model or fixed effect model was carried out according to the clinical heterogeneity. Meta-regression and subgroup analysis were carried out to detect the sources of heterogeneity. The publication bias in the present study was evaluated by Funnel plot, Egger's test and Begg's test. P value less than 0.05 was considered to be statistically significant. The statistical analyses in the current study were carried out by STATA version 12.0 software (Stata Corporation, College Station, TX, USA).

Results

Literature search results

The literature search in the present study identified 335 potential articles, involving 186 duplicate articles. After

Table 1 Characteristics of studies included in present meta analysis

Author/Country/Year	Detection method	Quantitative method	Antibody	Cutoff value (%)	Patient number (*n*)	High expression (%)	Mean age (range)	Pathologic type	Stage	Therapy	NOS value
Felix [5] America 2015	Tissue microarrays	Semi-quantitative	CINtec PLUS Kit	10	173	64.2	(20–74)	OC	I–IV	S	6
Hua [6] China 2015	Microscope (10 high-power fields/500 cells)	Semi-quantitative	Mouse monoclonal	50	119	49.6	NR	EOC	I–IV	S	7
Deng [7] China 2015	Microscope (5 high-power fields/1,000 cells)	Semi-quantitative	Mouse monoclonal	50.7	119	49.6	50 (32–78)	EOC	I–IV	S	6
Battista [8] Germany 2014	NR	Semi-quantitative	Monoclonal	20	108	73.8	61.7±11.4	EOC	I–IV	S	7
Vallen [9] Netherlands 2012	NR	Semi-quantitative	Anti-mouse/rabbit/rat-igg	20	61	65.6	55.6 (23–90)	OC	I–IV	S	6
Kim [10] Korea 2012	Tissue microarrays	Semi-quantitative	Mouse monoclonal	5	47	NR	53 (33–78)	EOC	III–IV	S	6
Lu et al. [11] China 2012	Microscope (5 high-power fields/1000 cells)	Quantitative	Mouse monoclonal	35	46	39.1	NR	OC	III–IV	S	8
Lee et al. [12] America 2005	Microscope (10 high-power fields/1000 cells)	Quantitative	NR	36.5	54	NR	NR	EOC	I–IV	S	7
Khalifeh [14] America 2005	Microscope (1000 cells)	Quantitative	NR	15	118	84.7	63 (38–89)	SOC	III–IV	S	6
Ali-Fehmi [15] America 2005	Microscope (1000 cells)	Quantitative	NR	Median	118	50.8	NR	SOC	III–IV	S	7
Nakayama [1] Japan 2004	Microscope (1000 cells)	Quantitative	Anti-human monoclonal	26	41	50.0	NR	OC	I–III	S	7
Layfield [18] America 1997	Microscope (15 high-power fields/1000–2000 cells)	Quantitative	Monoclonal	15	50	70.0	60 (30–80)	OC	III –IV	S	7
Wang [22] China 2016	Microscope (5 high-power fields)	Semi-quantitative	NR	25	242	61.2	NR	EOC	I–IV	S+C	6
Khandakar [23] India 2014	Microscope (500 cells)	Semi-quantitative	Monoclonal	50	62	11.3	50	SOC	III–IV	S+C	7
Bachmayr-Heyda [24] Australia 2013	Tissue microarrays	Quantitative	Mouse monoclonal	5	203	93.6	56 (18–85)	EOC	II–IV	S+C	6
Heeran [25] Denmark 2013	Microscope (500 cells)	Quantitative	Mouse monoclonal	10	606	51.0	59 (26–80)	EOC	I–IV	S+C	6

Table 1 (continued)

Author/Country/Year	Detection method	Quantitative method	Antibody	Cutoff value (%)	Patient number (*n*)	High expression (%)	Mean age (range)	Pathologic type	Stage	Therapy	NOS value
Liu [26] China 2012	Microscope (10 high-power fields)	Semi-quantitative	NR	50	166	77.7	61 (30–88)	EOC	I–IV	S+C	7
Katagiri [27] Japan 2012	Tissue microarrays	Semi-quantitative	Mouse monoclonal	NR	60	50.0	NR	OC	I–IV	S+C	6
Aune [28] Norway 2011	Microscope (10 high-power fields/1000 cells)	Quantitative	Monoclonal	36.7	68	50.0	64 ±11	OC	I–IV	S+C	7
Kondoh [29] America 2010	Microscope (5 high-power fields)	Quantitative	Monoclonal	40.3	104	45.2	62 (35–80)	SOC	III–IV	S+C	7
Adams [30] America 2009	Microscope (10 high-power fields)	Quantitative	Mouseanti-human	25	134	30.6	62 (26–84)	SOC	III–IV	S+C	7
Kobel [31] Canada 2008	Tissue microarrays	Quantitative	NR	Median	500	50.0	58 ± 0.6	OC	I–III	S+C	6
Garcia-Velasco [32] Spain 2008	Microscope	Quantitative	NR	30	72	50.0	57 (28–82)	EOC	I–IV	S+C	7
Tetu [33] Canada 2008	NR	Quantitative	NR	20	158	47.5	61	SOC	III–IV	S+C	6
Khouja [34] Norway 2007	NR	Semi-quantitative	Monoclonal	10	171	84.2	54 (21–70)	OC	III	S+C	7
Yamamoto [35] Japan 2007	Tissue microarrays	Semi-quantitative	Monoclonal	Median	119	50.0	57	SOC	III–IV	S+C	7
Yamamoto [36] Japan 2007	Microscope (400 cells)	Quantitative	Monoclonal	43	67	50.0	54 (36–75)	OC	I–IV	S+C	6
Green [37] England 2006	Microscope (200 cells)	Quantitative	Mouse monoclonal	20	108	63.0	75	EOC	II–IV	S+C	7
Surowiak [38] Polang 2006	Microscope	Quantitative	Monoclonal	50	41	58.5	51	OC	I–III	S+C	7
Kritpracha [39] Thailand 2005	Microscope (1000 cells)	Quantitative	Monoclonal	11.9	105	50.0	54.7 ±11.2	EOC	III–IV	S+C	6
Elie [40] France 2004	Microscope	Quantitative	Monoclonal	30	164	NR	59 (23–74)	EOC	III–IV	S+C	7
Camilleri-Broet [41] France 2004	Microscope (3 high-power fields)	Quantitative	Monoclonal	30	117	50.0	59 (23–74)	OC	III–IV	S+C	6

Table 1 (continued)

Author/Country/Year	Detection method	Quantitative method	Antibody	Cutoff value (%)	Patient number (*n*)	High expression (%)	Mean age (range)	Pathologic type	Stage	Therapy	NOS value
Itamochi [42] Japan 2002	Microscope (500 cells)	Quantitative	Monoclonal	18	41	46.3	49.8 (31–67)	OC	III–IV	S+C	7
Sengupta [43] England 2000	Microscope	Quantitative	Mouse monoclonal	10	62	62.9	55.5 (29–78)	EOC	I–IV	S+C	7
Costa [44] America 1999	Microscope (4 high-power fields/100 cells)	Quantitative	NR	44	117	55.6	58 (17–86)	OC	I–IV	S+C	7
Anttila [45] Finland 1998	Microscope (10 high-power fields)	Quantitative	Monoclonal	20	304	71.1	62 (18–85)	EOC	I–IV	S+C	7
Viale [46] Italy 1997	Microscope (2000 cells)	Quantitative	Monoclonal	30	59	50.0	NR	OC	I–IV	S+C	6
Masoumi-Moghaddam [47] Australia 2015	Microscope	Quantitative	Mouse monoclonal	10	100	60.0	NR	EOC	I–IV	S+C	6

NR not reported, *OS* overall survival, *DFS* disease free survival, *S* surgery, *C* chemotherapy, *R* radiotherapy, *EOC* epithelial ovarian cancer, *SOC* serous ovarian cancer, *OC* ovarian cancer, *NOS* Newcastle–Ottawa Quality Assessment Scale

browsing the abstracts, 47 articles were filtered out according to the criteria of inclusion and exclusion. We identified 102 potential eligible studies for further full-text review and 64 studies were eliminated for inadequate survival information. Finally, 38 eligible studies were included in the current study [5–42]. The process of literature retrieval is shown in Fig. 1. Study quality assessment was carried out by the Newcastle–Ottawa Scale (NOS).

Study selection and characteristics

The clinical characteristics of 38 eligible studies are summarized in Table 1. The publication time was between 1997 and 2016. The patient number was between 41 and 606, with an average value of 133. The NOS scores of 38 eligible studies varied from 7 to 8, with an average value of 7.6. The Ki-67 expression in the current study was measured in cancer tissues.

Prognostic significance of high Ki-67 expression

A total of 5004 OC patients from 38 eligible studies were included and analyzed (Fig. 2). The pooled hazard ratio (HR) was 1.35(95% CI 1.24–1.46, $P = 0.001$) for overall survival (OS) in OC patients.

Publication bias

The funnel plot (Fig. 3) showed obvious asymmetry. Egger's test ($P = 0.001$) found significant publication bias, whereas Begg's test ($P = 0.477$) did not find significant publication bias.

Contour-enhanced funnel plot

Contour-enhanced funnel plot with trim-and-fill method has been proposed as a valuable assessment method for the potential reasons of funnel plot asymmetry [44]. Contour lines with statistical significance of 0.01, 0.05, and 0.1 are drawn in the funnel plot. The new supplementary studies lie in the interval with non-statistical significance demonstrates that the funnel plot asymmetry may be caused by publication bias.

We drew a contour-enhanced funnel plot with trim-and-fill method to further study the potential reasons of funnel plot asymmetry (Fig. 4). In contour-enhanced funnel plot, green dot represented the original study and red triangle represented the new supplementary study. The contour-enhanced funnel plot supplement 11 new dummy studies and nine supplement studies (red triangle) were in the area with statistical significance, indicating that the publication bias in the current study might not be the major reason of funnel plot asymmetry.

Meta-regression and subgroup analysis

Subgroup analysis and meta-regression were carried out to study the sources of clinical heterogeneity. In subgroup analysis (Table 2), the pooled HRs were 1.66 (95% CI 1.34–2.05, $I^2=55.7\%$, $P=0.008$),1.43 (95% CI 0.86–2.36, $I^2=90.2\%$, $P=0.001$) and 1.11 (95% CI 1.03–1.20, $I^2=74.5\%$, $P=0.001$) for epithelial ovarian cancer (EOC) subgroup, serous ovarian cancer (SOC) subgroup and OC subgroup, suggesting that pathologic type might contribute to the clinical heterogeneity in the current study. Meta-regression analysis indicated that treatment might be a potential reason for clinical heterogeneity ($P=0.018$) in the present study.

Sensitivity analysis

To explore the actual influence of single study to the results, sensitivity analysis was carried out by removing single study every time.

The pooled HRs of sensitivity analysis ranged from 1.27 (95%CI: 1.17–1.37) to 1.45 (95%CI: 1.24–1.70) for OS, demonstrating that the pooled HR was not significantly impacted by any individual study (Table 3).

Cumulative meta-analysis

We carried out the cumulative meta-analysis to further explore the performance of Ki-67 expression for overall survival (Fig. 5). The pooled HRs ranged from 1.20 to 1.82

Fig. 1 Flowchart of study selection in the present study

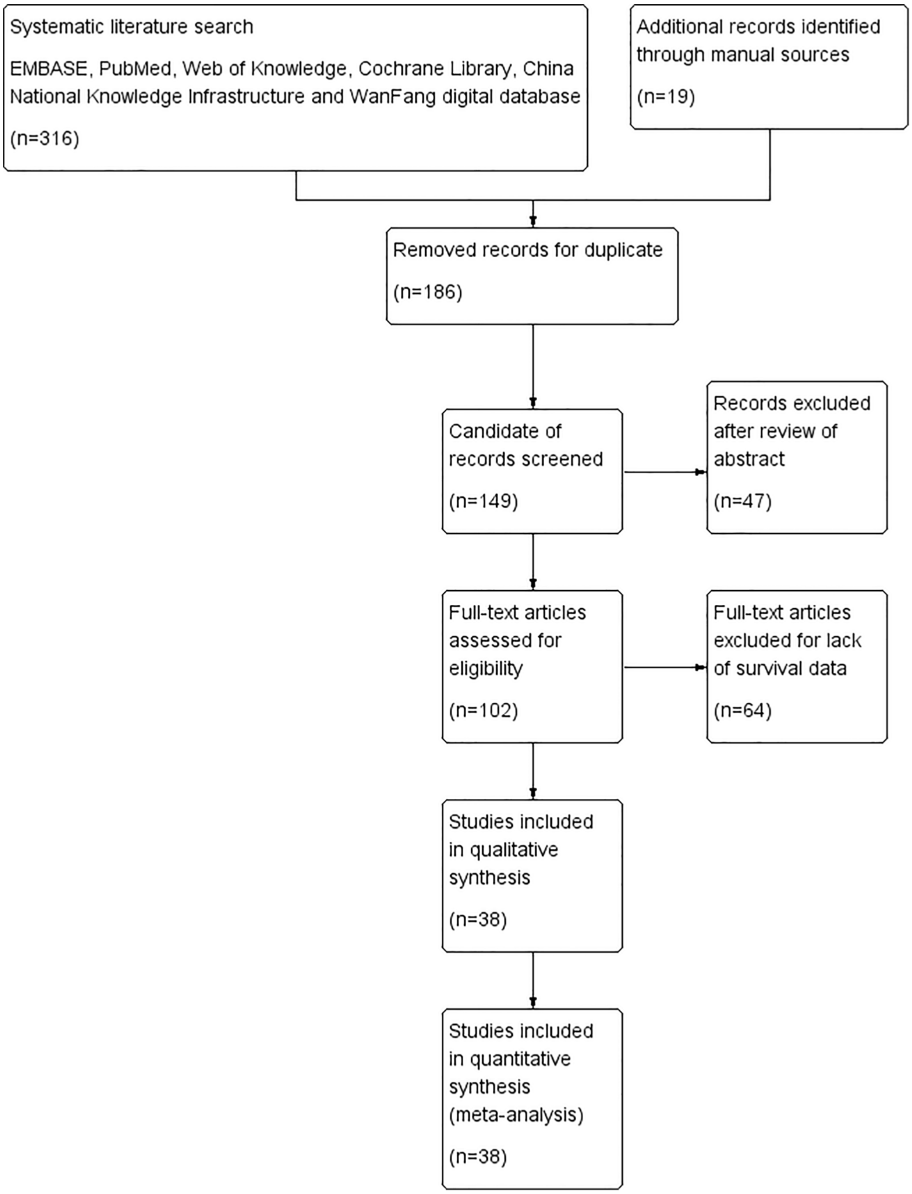


Fig. 2 Forest plot diagrams for correlation between Ki-67 expression and overall survival

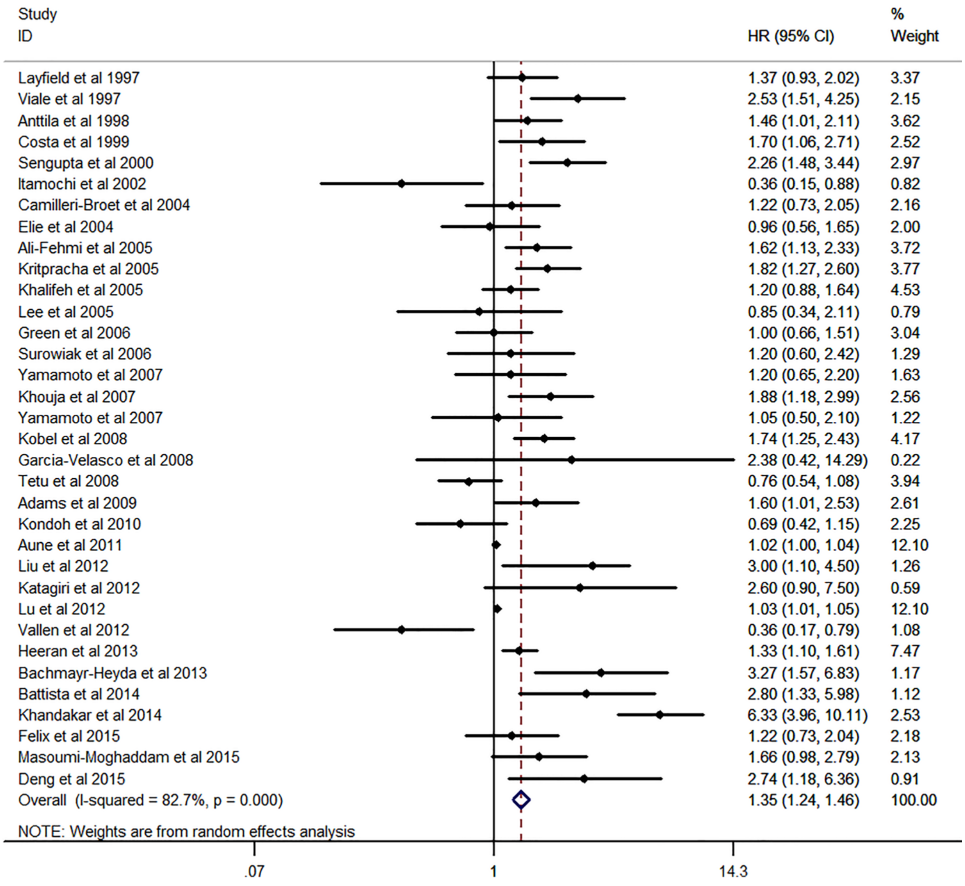
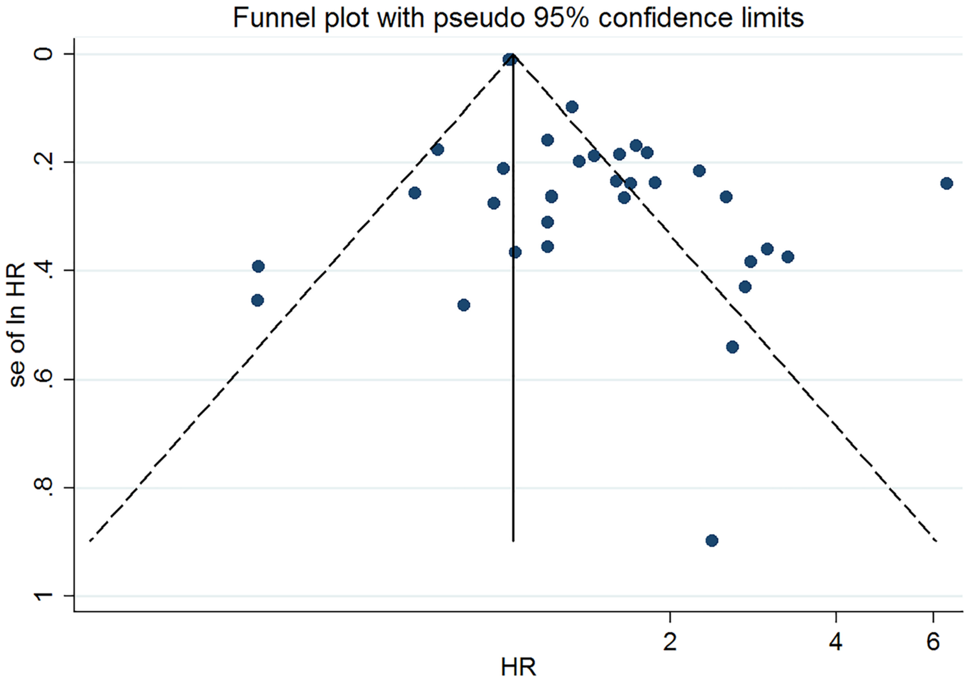


Fig. 3 Funnel plot of Ki-67 expression for overall survival



and all lower values of 95% CI were higher than 1.07 since 1998, demonstrating that the association between high Ki-67 expression and poor overall survival in OC patients was stable and reliable.

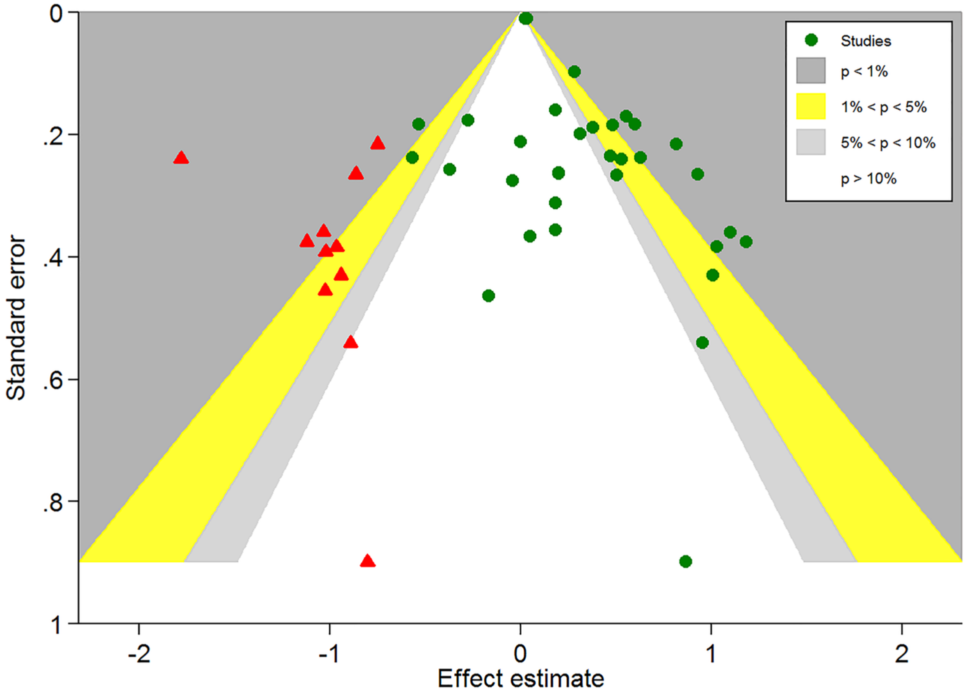


Fig. 4 Contour-enhanced funnel plot using the trim-and-fill method

Further exploration of significance and mechanism of Ki-67

To further explore the potential molecular biology pathogenic mechanism of Ki-67 for OC patients, we searched the Ki-67 (MKI67) by the Search Tool for the Retrieval of Interacting Genes (STRING, https://string.embl.de/) database and constructed the protein–protein interaction network

Table 2 Subgroup analysis for association between Ki-67 expression and overall survival in ovarian cancer patients

Term	Subgroup	Numberof study	Pooled HR	95% CI	*P*value	Heterogeneity I^2 (%)	*P*value
Location	Asian	9	1.711	1.038–2.819	0.035	76.5	0.001
	Non-Asian	25	1.345	1.162–1.556	0.001	81.8	0.001
Cut off value	≥ 40%	10	1.743	1.179–2.576	0.005	82	0.001
	< 40%	23	1.198	1.109–1.296	0.001	76.8	0.001
Patient number	≥ 100	21	1.436	1.237–1.667	0.001	58.8	0.001
	< 100	13	1.212	1.086–1.353	0.001	88.2	0.001
High expression rate	≥ 50%	25	1.504	1.284–1.761	0.001	78.9	0.001
	< 50%	7	1.291	0.782–2.133	0.318	92.2	0.001
Pathologic type	EOC	13	1.660	1.343–2.051	0.001	55.7	0.008
	SOC	7	1.429	0.864–2.364	0.164	90.2	0.001
	OC	14	1.110	1.029–1.198	0.007	74.5	0.001
Stage	III–IV	12	1.283	0.922–1.787	0.139	85.2	0.001
	I–IV	22	1.278	1.173–1.392	0.001	79.1	0.001
Treatment	S	1	1.249	0.976–1.600	0.077	71.8	0.001
	S + C	25	1.499	1.244–1.806	0.001	85.2	0.001
Extraction method	Curve	10	1.624	1.171–2.252	0.004	82.9	0.001
	Reported	24	1.194	1.101–1.295	0.001	75.9	0.001
Analysis method	Multivariate	12	1.352	1.019–1.794	0.037	85.1	0.001
	Univariate	34	1.350	1.240–1.460	0.001	82.7	0.001

HR hazard ratio, *CI* confidence interval, *S* surgery, *C* chemotherapy, *EOC* epithelial ovarian cancer, *SOC* serous ovarian cancer, *OC* ovarian cancer

Table 3 Influence analysis of single study to the pooled HRs of Ki-67 expression for overall survival

Study omitted	HR	Lower value of 95% CI	Upper value of 95% CI
1	1.345582	1.235876	1.465026
2	1.32374	1.217909	1.438769
3	1.341728	1.232387	1.460771
4	1.337223	1.229021	1.454952
5	1.320239	1.214499	1.435185
6	1.35921	1.250361	1.477536
7	1.349935	1.240328	1.469227
8	1.356475	1.246383	1.476291
9	1.335115	1.226638	1.453184
10	1.327257	1.219942	1.444013
11	1.35445	1.243159	1.475704
12	1.351963	1.242926	1.470566
13	1.359883	1.248912	1.480715
14	1.349045	1.239967	1.467719
15	1.3496	1.240293	1.468539
16	1.332686	1.225133	1.44968
17	1.351247	1.242003	1.470101
18	1.328193	1.220544	1.445336
19	1.344943	1.236877	1.46245
20	1.377825	1.265332	1.500318
21	1.339476	1.230916	1.45761
22	1.366845	1.256144	1.487302
23	1.451573	1.241637	1.697006
24	1.329619	1.223389	1.445073
25	1.340524	1.232909	1.457531
26	1.451063	1.239578	1.698629
27	1.362967	1.25396	1.481449
28	1.346384	1.234718	1.468149
29	1.329009	1.222974	1.444238
30	1.333043	1.226309	1.449067
31	1.266743	1.173077	1.367888
32	1.349958	1.24034	1.469264
33	1.33976	1.231398	1.457656
34	1.336108	1.229002	1.452548
Combined	1.3465667	1.2385109	1.46405

HR hazard ratio, *CI* confidence interval

(PPI). This protein–protein interaction (Fig. 6) network was generated using methods of experiment, neighborhood, co-occurrence, gene fusion, co-expression, and text mining. There were 11 nodes, 39 edges in this protein–protein interaction network, with average node degree of 7.09 and PPI enrichment *P* value of 7.92e-06. Among ten relevant genes, the top five node degree genes were MKI67IP (Combined score 0.981), GTPBP10 (Combined score 0.865), CDK1 (Combined score 0.856), CCNA2 (Combined score 0.837), and KIF15 (Combined score 0.834).

Prognostic role of Ki-67 gene for overall survival

To explore the prognostic role of Ki-67 gene for overall survival, the survival curve of Ki-67 (using gene symbol: MIK67/probe ID: 212022_s_at) and log-rank analysis were performed from KM plotter (https://kmplot.com/analysis/). The OC patients analyzed in KM plotter were from the Gene Expression Omnibus (GEO) database and The Cancer Genome Atlas (TCGA) database. There were 1656 OC patients come from TCGA database ($n = 565$), GSE14764 ($n = 80$), GSE15622 ($n = 35$), GSE18520 ($n = 63$), GSE19829 ($n = 28$), GSE23554 ($n = 28$), GSE26193($n = 107$), GSE26712($n = 195$), GSE27651($n = 49$), GSE30161($n = 58$), GSE51373 ($n = 28$), GSE63885 ($n = 101$),and GSE9891 ($n = 285$). As shown in Fig. 7, the overall survival rate of patients in high Ki-67 expression was poorer than that in patients in low Ki-67 expression ($P < 0.001$). The pooled hazard ratio (HR) was 1.30 (95% CI 1.12–1.521) for overall survival in OC patients.

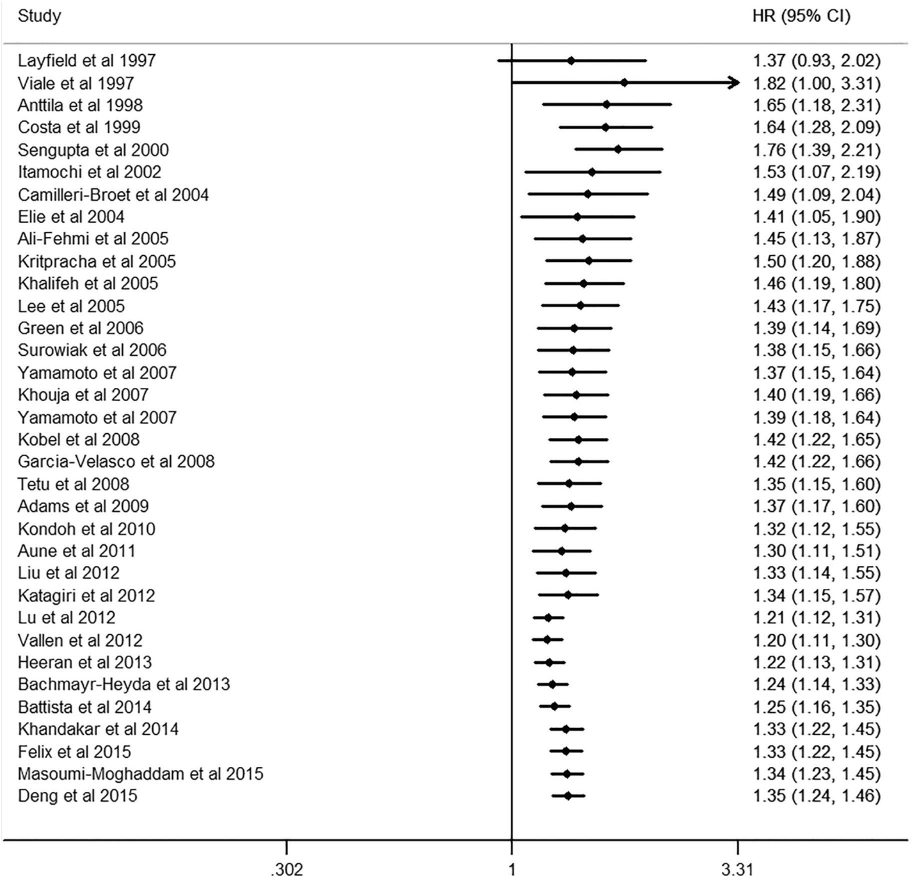
Discussion

The present meta-analysis, which included 5004 ovarian patients from 38 eligible studies, suggested that high Ki-67 expression is significantly associated with poor overall survival in OC patients. Subgroup analysis found significant association between high ki-67 expression and poor overall survival in most subgroup analysis, especially for patients in Asian group (HR 1.71) and epithelial ovarian cancer group (HR 1.66). Sensitivity analysis and cumulative meta-analysis demonstrated that the association between high Ki-67 expression and poor OS in OC patients was stable and reliable.

Several studies have reported that high Ki-67 expression was associated with poor OS in OC patients [7, 8, 14]. These studies revealed that high Ki-67 expression had a predictive value for poor prognosis in patients with OC. The conclusion of the present meta-analysis was consistent with that of these previous studies [7, 8, 14]. Several original studies and meta-analysis have reported that there was a significant association between high Ki-67 expression and poor prognosis in patients with non-small cell lung cancer, cervical cancer and gastrointestinal stromal tumor [45–47].

The heterogeneity was significant in the current study. There might be several reasons for clinical heterogeneity: Firstly, the clinical heterogeneity in the current study might be caused by different cutoff values of Ki-67 expression in various studies. Secondly, subgroup analysis indicated that pathologic type might be a potential source of clinical

Fig. 5 Cumulative meta-analysis of association of Ki-67 expression for overall survival in ovarian cancer patients



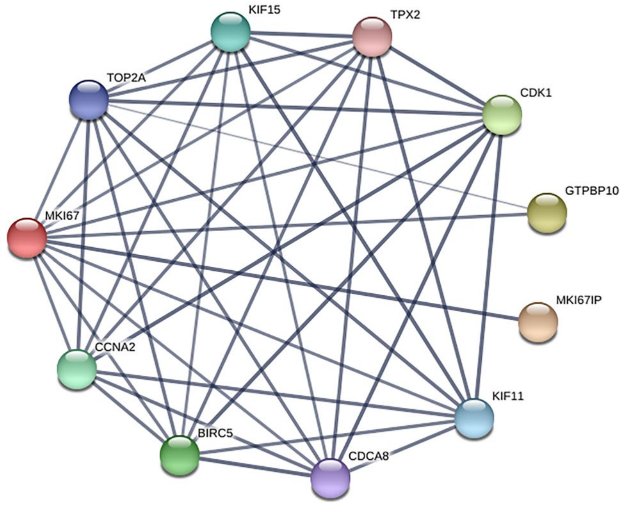


Fig. 6 Protein–protein interaction network of Ki-67 and other relevant protein

heterogeneity. Thirdly, meta-regression indicated that treatment might contribute to clinical heterogeneity.

There were several potential sources of funnel plot asymmetry in the current study: Firstly, publication bias might be one important reason of funnel plot asymmetry. Secondly, poor methodological quality in some studies might be another potential reason for funnel plot asymmetry. Thirdly, true heterogeneity in intervention effects might result in funnel plot asymmetry.

We performed a contour-enhanced funnel plot to study the potential reasons of funnel plot asymmetry. The contour-enhanced funnel plot demonstrated that 9 of the 11 supplementary studies were in areas with statistical significance, indicating that the publication bias in the current study might not be the major reason of funnel plot asymmetry. Further sensitivity analysis and cumulative meta-analysis demonstrated that the association between high Ki-67 expression and poor OS in OC patients was stable and reliable.

The present meta-analysis has three advantages as follows: First, the present study finally included 38 eligible studies and 5004 OC patients, which could significantly strengthen the persuasiveness of the study results. Second, detection methods of Ki-67 expression in 38 eligible studies were all IHC. Third, studies published in Chinese were also enrolled in the current study as English literature, increasing the representation of study cohort. Fourth, IHC method for Ki-67 expression could be carried out through microscope and was a simple feasible detection method for clinical practice. IHC method for Ki-67 expression was of great

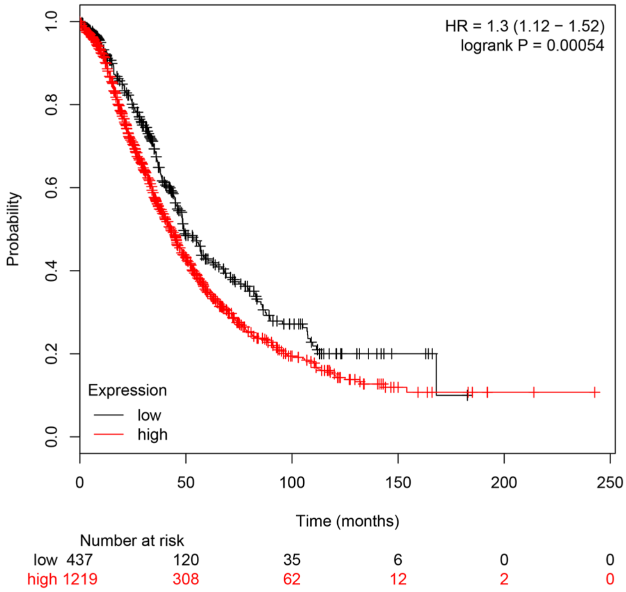


Fig. 7 Survival curve of Ki-67 gene for overall survival

importance for developing countries where detailed pathologic diagnosis might not be available in clinical practice.

The results in the current meta-analysis need to be interpreted cautiously for several shortcomings. Firstly, high Ki-67 expression was defined according to different cut-off values in various studies. Secondly, clinical heterogeneity was inevitable due to different baseline characteristics such as tumor stage, treatment and race. Thirdly, some data were indirectly extracted from Kaplan–Meier survival curves. Although this extract method from original Kaplan–Meier survival curves has been widely utilized for survival analysis, the sources of information inaccuracy could not be completely eliminated in extracting survival information from Kaplan–Meier survival curves.

Conclusion

In conclusion, high Ki-67 expression is significantly associated with poor overall survival and may serve as a prognostic biomarker for ovarian cancer patients.

Author contributions Conceptualization: ZZ, DQ, WC. Data curation: ZZ, WC. Formal analysis: ZZ, WC. Funding acquisition: ZZ. Investigation: ZZ, WC, DQ. Methodology: ZZ, WC, DQ. Project administration: ZZ, DZ. Resources: ZZ, HL. Software: ZZ, DZ, HL. Supervision: ZZ, WC. Validation: ZZ, DQ,WC. Visualization: ZZ, HL. Writing - original draft: ZZ, WC. Writing- review& editing: ZZ, WC.

Funding This study was funded in part by Health Department and Finance Department of Guangdong Province. The Grant Numbers were No: B2018237 (Grant Recipient: Zhiqiao Zhang) and No: A2016450 (Grant Recipient: Zhiqiao Zhang).The total funding account was RMB 5000. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The URLs of Health Department of Guangdong Province was https://www.gdwst.gov.cn/.

Compliance with ethical standards

Conflict of interest The authors have declared that they had no conflict of interests in relation to the work described. The authors have declared that they have had full control of all primary data and that they agreed to allow the Journal to review their data if requested.

Ethical approval The present study is a meta-analysis and ethical approval is not needed for meta-analysis.

References

1. Siegel RL, Miller KD, Jemal A (2017) Cancer statistics, 2017. CA Cancer J Clin 67:7–30
2. Bast RC Jr, Hennessy B, Mills GB (2009) The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 9:415–428
3. Schluter C, Duchrow M, Wohlenberg C, Becker MH, Key G, Flad HD et al (1993) The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. J Cell Biol 123:513–522
4. Cattoretti G, Becker MH, Key G, Duchrow M, Schluter C, Galle J et al (1992) Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 168:357–363
5. Felix AS, Sherman ME, Hewitt SM, Gunja MZ, Yang HP, Cora RL et al (2015) Cell-cycle protein expression in a population-based study of ovarian and endometrial cancers. Front Oncol 5:25
6. Hua M, Yan S, Deng Y, Xi Q, Liu R, Yang S et al (2015) CAP1 is overexpressed in human epithelial ovarian cancer and promotes cell proliferation. Int J Mol Med 35:941–949
7. Deng Y, Chen C, Hua M, Xi Q, Liu R, Yang S et al (2015) Annexin A2 plays a critical role in epithelial ovarian cancer. Arch Gynecol Obstet 292:175–182
8. Battista MJ, Mantai N, Sicking I, Cotarelo C, Weyer V, Lebrecht A et al (2014) Ki-67 as an independent prognostic factor in an unselected cohort of patients with ovarian cancer: results of an explorative, retrospective study. Oncol Rep 31:2213–2219
9. Vallen MJ, Massuger LF, Ten DG, Bulten JV, Kuppevelt TH (2012) Highly sulfated chondroitin sulfates, a novel class of prognostic biomarkers in ovarian cancer tissue. Gynecol Oncol 127:202–209
10. Kim H, Kim K, No JH, Jeon YT, Jeon HW, Kim YB (2012) Prognostic value of biomarkers related to drug resistance in patients with advanced epithelial ovarian cancer. Anticancer Res 32:589–594
11. Lu M, Xiang J, Xu F, Wang Y, Yin Y, Chen D (2012) The expression and significance of pThr32-FOXO3a in human ovarian cancer. Med Oncol 29:1258–1264
12. Lee JS, Choi YD, Choi C, Lee MC, Park CS, Min KW (2005) Expression of PTEN in ovarian epithelial tumors and its relation to tumor behavior and growth. Anal Quant Cytol Histol 27:202–210
13. Khalifeh I, Munkarah AR, Schimp V, Morris R, Lawrence WD, Ali-Fehmi R (2005) The impact of c-kit and ki-67 expression on

patients prognosis in advanced ovarian serous carcinoma. Int J Gynecol Pathol 24:228–234

14. Ali-Fehmi R, Morris RT, Bandyopadhyay S, Che M, Schimp V, Malone JM et al (2005) Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma: correlation with tumor cell proliferation, apoptosis, angiogenesis, and survival. Am J Obstet Gynecol 192:819–825
15. Nakayama K, Takebayashi Y, Hata K, Fujiwaki R, Iida K, Fukumoto M et al (2004) Allelic loss at 19q12 and Xq11-12 predict an adverse clinical outcome in patients with mucinous ovarian tumours of low malignant potential. Br J Cancer 90:1204–1210
16. Layfield LJ, Saria EA, Berchuck A, Dodge RK, Thompson JK, Conlon DH et al (1997) Prognostic value of MIB-1 in advanced ovarian carcinoma as determined using automated immunohistochemistry and quantitative image analysis. J Surg Oncol 66:230–237
17. Wang K, Li D, Sun L (2016) High levels of EGFR expression in tumor stroma are associated with aggressive clinical features in epithelial ovarian cancer. Onco Targets Ther 9:377–386
18. Masoumi-Moghaddam S, Amini A, Wei AQ, Robertson G, Morris DL (2015) Sprouty 1 predicts prognosis in human epithelial ovarian cancer. Am J Cancer Res 5:1531–1541
19. Khandakar B, Mathur SR, Kumar L, Kumar S, Datta Gupta S, Iyer VK et al (2014) Tissue biomarkers in prognostication of serous ovarian cancer following neoadjuvant chemotherapy. Biomed Res Int 401245
20. Bachmayr-Heyda A, Aust S, Heinze G, Polterauer S, Grimm C, Braicu EI et al (2013) Prognostic impact of tumor infiltrating CD8+ T cells in association with cell proliferation in ovarian cancer patients–a study of the OVCAD consortium. BMC Cancer 13:422
21. Heeran MC, Hogdall CK, Kjaer SK, Christensen L, Jensen A, Blaakaer J et al (2013) Prognostic value of tissue protein expression levels of MIB-1 (Ki-67) in Danish ovarian cancer patients. From the 'MALOVA' ovarian cancer study. APMIS 121:1177–1186
22. Liu P, Sun YL, Du J, Hou XS, Meng H (2012) CD105/Ki67 coexpression correlates with tumor progression and poor prognosis in epithelial ovarian cancer. Int J Gynecol Cancer 22:586–592
23. Katagiri A, Nakayama K, Rahman MT, Rahman M, Katagiri H, Nakayama N et al (2012) Loss of ARID1A expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. Mod Pathol 25:282–288
24. Aune G, Stunes AK, Tingulstad S, Salvesen O, Syversen U, Torp SH (2011) The proliferation markers Ki-67/MIB-1, phosphohistone H3, and survivin may contribute in the identification of aggressive ovarian carcinomas. Int J Clin Exp Pathol 4:444–453
25. Kondoh E, Mori S, Yamaguchi K, Baba T, Matsumura NC, Barnett J et al (2010) Targeting slow-proliferating ovarian cancer cells. Int J Cancer 126:2448–2456
26. Adams SF, Levine DA, Cadungog MG, Hammond R, Facciabene A, Olvera N et al (2009) Intraepithelial T cells and tumor proliferation: impact on the benefit from surgical cytoreduction in advanced serous ovarian cancer. Cancer 115:2891–2902
27. Kobel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C et al (2008) Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med V5:e232
28. Garcia-Velasco A, Mendiola C, Sanchez-Munoz A, Ballestin C, Colomer R, Cortes-Funes H (2008) Prognostic value of hormonal receptors, p53, ki67 and HER2/neu expression in epithelial ovarian carcinoma. Clin Transl Oncol 10:367–371
29. Tetu B, Popa I, Bairati I, L'Esperance S, Bachvarova M, Plante M et al (2008) Immunohistochemical analysis of possible chemoresistance markers identified by micro-arrays on serous ovarian carcinomas. Mod Pathol 21:1002–1010
30. Khouja MH, Baekelandt M, Nesland JM, Holm R (2007) The clinical importance of Ki-67, p16, p14, and p57 expression in patients with advanced ovarian carcinoma. Int J Gynecol Pathol 26:418–425
31. Yamamoto S, Tsuda H, Kita T, Maekawa K, Fujii K, Kudoh K et al (2007) Clinicopathological significance of WT1 expression in ovarian cancer: a possible accelerator of tumor progression in serous adenocarcinoma. Virchows Arch 451:27–35
32. Yamamoto S, Tsuda H, Yoshikawa T, Kudoh K, Kita T, Furuya K et al (2007) Clear cell adenocarcinoma associated with clear cell adenofibromatous components: a subgroup of ovarian clear cell adenocarcinoma with distinct clinicopathologic characteristics. Am J Surg Pathol 31:999–1006
33. Green JA, Berns EM, Coens C, Luijk I, Thompson-Hehir JV, Diest P et al (2006) Alterations in the p53 pathway and prognosis in advanced ovarian cancer: a multi-factorial analysis of the EORTC Gynaecological Cancer group (study 55865). Eur J Cancer 42:2539–2548
34. Surowiak P, Materna V, Kaplenko I, Spaczynski M, Dietel M, Lage H et al (2006) Topoisomerase 1A, HER/2neu and Ki67 expression in paired primary and relapse ovarian cancer tissue samples. Histol Histopathol 21:713–720
35. Kritpracha K, Hanprasertpong J, Chandeying V, Dechsukhum C, Geater A (2005) Survival analysis in advanced epithelial ovarian carcinoma in relation to proliferative index of MIB-1 immunostaining. J Obstet Gynaecol Res 31:268–276
36. Elie C, Geay JF, Morcos M, Tourneau A, Girre V, Broet P et al (2004) Lack of relationship between EGFR-1 immunohistochemical expression and prognosis in a multicentre clinical trial of 93 patients with advanced primary ovarian epithelial cancer (GINECO group). Br J Cancer 91:470–475
37. Camilleri-Broet S, Hardy-Bessard AC, Le Tourneau A, Paraiso D, Levrel O, Leduc B et al (2004) HER-2 overexpression is an independent marker of poor prognosis of advanced primary ovarian carcinoma: a multicenter study of the GINECO group. Ann Oncol 15:104–112
38. Itamochi H, Kigawa J, Sugiyama T, Kikuchi Y, Suzuki M, Terakawa N (2002) Low proliferation activity may be associated with chemoresistance in clear cell carcinoma of the ovary. Obstet Gynecol 100:281–287
39. Sengupta PS, McGown AT, Bajaj V, Blackhall F, Swindell R, Bromley M et al (2000) P53 and related proteins in epithelial ovarian cancer. Eur J Cancer 36:2317–2328
40. Costa MJ, Hansen CL, Walls JE, Scudder SA (1999) Immunohistochemical markers of cell cycle control applied to ovarian and primary peritoneal surface epithelial neoplasms: p21(WAF1/CIP1) predicts survival and good response to platinin-based chemotherapy. Hum Pathol 30:640–647
41. Anttila M, Kosma VM, Ji H, Wei-Ling X, Puolakka J, Juhola M et al (1998) Clinical significance of alpha-catenin, collagen IV, and Ki-67 expression in epithelial ovarian cancer. J Clin Oncol 16:2591–2600
42. Viale G, Maisonneuve P, Bonoldi E, Bacco A, Bevilacqua P, Panizzoni GA et al (1997) The combined evaluation of p53 accumulation and of Ki-67 (MIB1) labelling index provides independent information on overall survival of ovarian carcinoma patients. Ann Oncol V8:469–476
43. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D et al (2000) Meta analysis of observational studies in epidemiology: a proposal for reporting. Meta analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 283:2008–2012
44. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L (2008) Contour-enhanced meta analysis funnel plots help distinguish publication bias from other causes of asymmetry. J Clin Epidemiol 61:991–996

45. Wen S, Zhou W, Li CM, Hu J, Hu XM, Chen P et al (2015) Ki-67 as a prognostic marker in early-stage non-small cell lung cancer in Asian patients: a meta analysis of published studies involving 32 studies. BMC Cancer 15:520
46. Pan D, Wei K, Ling Y, Su S, Zhu M, Chen G (2015) The prognostic role of Ki-67/MIB-1 in cervical cancer: a systematic review with meta analysis. Med Sci Monit 21:882–889
47. Pyo JS, Kang G, Sohn JH (2016) Ki-67labeling index can be used as a prognostic marker in gastrointestinal stromal tumor: asystematic review and meta analysis. Int J Biol Markers 31:204–210