Original Article
Clinicopathological significance and prognostic value of signal transducer and activator of transcription 3 (STAT3) and phospho-STAT3 expression in breast cancer: a meta-analysis of 4031 cases

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Abstract: Background: The clinicopathological significance and prognostic value of signal transducer and activator of transcription 3 (STAT3) and phospho-STAT3 (p-STAT3) in breast cancer remains controversial. A meta-analysis was carried out to quantitatively assess the impact of STAT3 and p-STAT3 on clinicopathological features and survival in breast cancer. Methods: Published studies were searched in PubMed, Google Scholar, Web of Science, Wiley Online Library, Cochrane Central Register of Controlled Trials, Science Direct, EMBASE, Chinese CNKI, Wan Fang, Ovid and LILACS up to 15th April 2016. Correlation between STAT3 or p-STAT3 expression and clinical parameters and survival data was extracted after the literatures were screened. Meta-analysis was conducted with Stata 12.0 and pooled odds ratio (OR) or hazard ratios (HR) with 95% confidence interval (CI) was calculated. Results: A total of 24 references were included with 4,031 patients. The combined ORs suggested that overexpression of STAT3 and p-STAT3 was remarkably associated with higher histological grade (OR=2.02; 95% CI: 1.39-2.94; *P*<0.001), lymph node metastases (LNM) (OR=3.00; 95% CI: 2.25-3.99; *P*<0.001) and TNM stage (OR=4.03; 95% CI: 3.07-5.27; *P*<0.001) in breast cancer patients. Furthermore, pooled analysis of positive STAT3 and p-STAT3 expression demonstrated poor prognosis in East Asian population (HR=1.32; 95% CI: 1.02-1.72; *P*=0.036), while favorable in the non-East Asians (HR=0.57; 95% CI: 0.37-0.88; *P*=0.012). Conclusion: Overexpression of STAT3 and p-STAT3 is correlated with detrimental grade, LNM and TNM stage of breast cancer. High STAT3 and p-STAT3 expression seems to predict poor outcome in East Asians, while favorable prognosis could be found in non-East Asians.

Keywords: STAT3, p-STAT3, breast cancer, clinicopathological, prognostic

Introduction

Breast cancer, with high morbidity and mortality globally, is the most frequent carcinoma diagnosed among women [1]. Global breast cancer incidence increased between 1980 and 2010 with an annual increase rate of 3.1% [2]. A recent study has also indicated that breast cancer in America has accounted for 29% in new cancer cases among women [3]. Early symptoms in breast cancer patients are hidden with high misdiagnosis rate and poor prognosis. Approximately 5%-10% of breast cancer patients are already metastatic when diagnosed [4]. As a complex multifactorial disease, although many treatment strategies have been significantly improved, the recurrence rate and mortality still remain high [5]. With respect of the molecular markers in the monitor of cancer process, several molecular markers with prognostic significance were identified in recent years. For example, Bcl-2 [6] and p27 [7] were reported to be poor prognostic markers in breast cancer. However, insufficient molecular markers are clinically applicable to recognize the progress and prognosis of breast cancer.

Individualized treatment also prompts the study and translation of specific prognostic biomarkers. Hence, it is crucial to identify significant clinicopathological and prognostic markers to assist achieve satisfactory prognosis for breast cancer patients.

STAT3 is a member of signal transducer and activator of transcription (STAT) with critical roles in oncogenesis in the cytoplasm [8]. STAT3 molecules can be activated by tyrosine kinase signals and dimerized to phospho-STAT3 (p-STAT3), which regulates the expression of genes in the nucleus [9]. STAT3 and p-STAT3 can bring about cell proliferation, invasion, angiogenesis, as well as metastasis [10, 11]. Up to date, a series of studies have reported that STAT3 and p-STAT3 are associated with clinical significance and prognosis. Xu et al [12] found that overexpression of STAT3 or p-STAT3 was a poor prognostic indicator among non-small-cell lung cancer (NSCLC) on the basis of meta-analysis. In the research of Chen et al, high STAT3 expression was correlated with poor prognosis and many clinical parameters in gastric cancer [13]. In digestive system cancers, p-STAT3 was revealed to be a poor prognostic marker in the meta-analysis of Li et al [14].

A growing number of studies in breast cancer have been performed and identified the association of STAT3 or p-STAT3 expression with clinicopathological and prognostic significance, however, the conclusion remains controversial [15-38]. Kong et al [39] found that high STAT3 expression but not p-STAT3 was related to poor outcome in tumor patients, with 4 breast cancer studies included in meta-analysis. Wu et al [40] reported positive STAT3 expression indicated poor prognosis in hepatic cancer, lung cancer, gastric cancer, gliomas, prostate cancer, osteosarcoma and pancreatic cancer, while interestingly, showed favorable prognosis in breast cancer (5 studies included) of meta-analysis. Current study was further conducted on a large data series of breast cancer to confirm the prognostic value of STAT3 and p-STAT3 in breast cancer, as well as the role of STAT3 or p-STAT3 in the progression of breast cancer.

Materials and methods

Literature search

PubMed, Google Scholar, Web of Science, Wiley Online Library, Cochrane Central Register of Controlled Trials, Science Direct, EMBASE, Chinese CNKI, Wan Fang, Ovid and LILACS were searched for relevant published studies up to 15th April, 2016. The combination of keywords were as follows: "signal transducer and activator of transcription 3" or "STAT3", "phospho-signal transducer and activator of transcription 3" or "p-STAT3" or "phospho-STAT3" AND "breast cancer" or "breast carcinoma" or "mammary cancer" or "breast neoplasms".
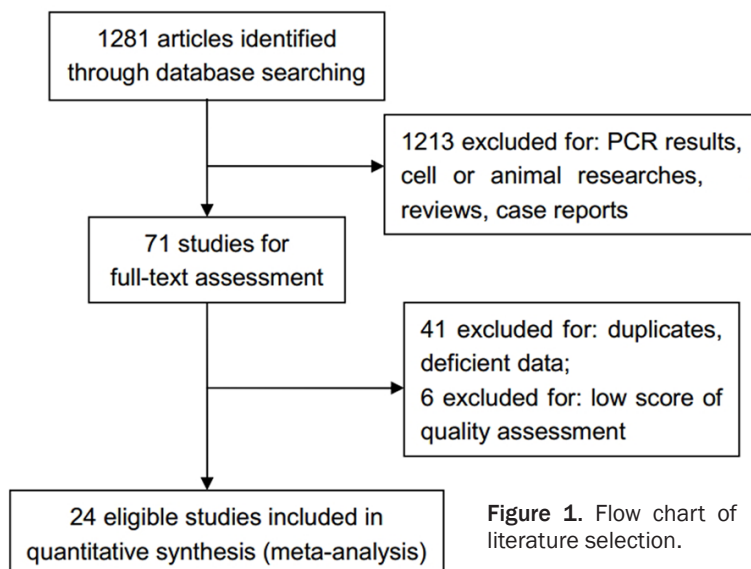
Selection criteria

Inclusion criteria included: (1) STAT3 or p-STAT3 expression were evaluated by immunohistochemistry (IHC); (2) primary human breast cancer tissues (not plasma or serum) were researched in articles; (3) the correlation between STAT3 or p-STAT3 and clinicopathological parameters or prognosis were included in papers; (4) studies provided the prognosis related hazard ratios (HR) together with 95% confidence interval (95% CI), or original survival data, survival curve, survival rate and *P* values to calculate the corresponding HR and 95% CI; (5) papers were published as a full text in English or Chinese. When multiple studies based on the same patient population, the latest published or the most complete studies were selected.

Exclusion criteria included: (1) reviews, abstracts, letters, case reports, and duplicate reports; (2) cell or animal studies; (3) studies of secondary breast cancer; (4) articles without sufficient clinicopathological or survival data for calculating odds ratio (OR) or HR and 95% CI; (5) experimental methods with Real-time PCR or Gene polymorphism; (6) low score of quality assessment.

Data extraction

Eligible studies were selected by two independent investigators (Chun-Yao Li and Lan-Shan Huang) according to the selection criteria above, and extracted data from all the articles. Controversial problems were discussed by two reviewers or assessed by the third investigator (Gang Chen). The following data was extracted from each study: (1) the first author and time of publication; (2) origin of patients, age, total number, human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER) and progesterone receptor (PR) status; (3) cut-off value of IHC for positive STAT3 or p-STAT3 expression,

1281 articles identified through database searching

1213 excluded for: PCR results, cell or animal researches, reviews, case reports

71 studies for full-text assessment

41 excluded for: duplicates, deficient data; 6 excluded for: low score of quality assessment

24 eligible studies included in quantitative synthesis (meta-analysis)

Figure 1. Flow chart of literature selection.

clinicopathological parameters and follow-up time; (4) HR and 95% CI of positive STAT3 or p-STAT3 expression and their overall survival (OS) or disease-free survival (DFS) rates were extracted. If HR did not reported directly, original survival data was extracted to calculate HR. If only survival curves were obtainable, data from the graphical survival plots were extracted through Engauge Digitizer version 4.1 (downloaded from http://digitizer.sourceforge.net/) and estimated the HR and 95% CI [41]. If insufficient data was reported, the author was contacted for unpublished results.

Quality assessment

The Newcastle-Ottawa Scale (NOS) criteria [42] was selected to evaluate the quality of included studies by two researchers (Xiao-Miao Lin and Rong-Quan He) independently for cohort and case-control studies with appropriate modifications. Scores ranged from 0 to 9 represents low to high and 6 scores or more indicating high quality.

Statistical analysis

ORs and 95% CIs were pooled to estimate the connection between STAT3 or p-STAT3 and clinicopathological parameters of breast cancer including age, tumor size (≥ 2/<2), histopathological grade (3/1+2), lymph node metastases (LNM), tumor node metastasis (TNM) stage (III+IV/I+II) and ER, PR, HER2 expression status. HRs was obtained to describe the correla-

tion between STAT3 or p-STAT3 expression levels and survival rates, and combined HRs with their 95% CIs to estimate their relationship quantitatively. The multivariate analysis of ORs and HRs were applied as far as possible to rule out other related factors on the effect of STAT3 or p-STAT3. Heterogeneity and inconsistency index (I^2) statistic were assessed by Chi-square (x^2) based Q statistical test in the meta-analysis when pooled ORs and HRs. Significant heterogeneity was defined if the $I^2 > 50\%$ or $P < 0.10$, the random-effects model was applied when calculate estimates, otherwise, the fixed-effects model was chosen. Subgroup analysis was performed to search potential heterogeneity sources that might distort the results. By convention, pooled OR (or HR)>1 indicated worse survival for the research group with positive STAT3 or p-STAT3 expression or the significant association of STAT3 or p-STAT3 with clinicopathological characteristics. If the 95% CI for pooled OR (or HR) did not overlap 1, it was considered as statistically significant.

The stability of merged results was assessed by sensitivity analysis. The publication bias was evaluated by quantitatively performing Egger's linear regression and visually assessing a funnel plot by Begg's tests. $P < 0.05$ was considered as significant publication bias statistically. All statistical calculations were performed via Stata version 12.0 (StataCorp, College Station, TX, USA).

Results

Search results

The flow chart of literature selections was shown in **Figure 1**. Totally, 1281 articles were achieved by the aforementioned search strategy including 943 articles in English and 338 studies in Chinese. According to selection criteria, 50 English papers and 21 Chinese papers were selected through title and abstract being checked. The remaining 71 articles were selected for full-text evaluation and 47 papers were

Table 1. Characteristics of included studies for clinicopathological features

First author	Year	Origin of popu-lation	Age (years)	Histology	Stage	Grade 3 (%)	Lymph node sta-tus (%)	Num-ber of patients	STAT3+ (%)	p-STAT3+ (%)	Method	Cut-off	Her2+ (%)	ER+ (%)	PR+ (%)	Quality score
Yeh	2006	China	31-83 (51.4±14.2)	Invasive ductal cancer	I-III	26 (38.8)	≥1 (51.5)	68	NR	C+N: 42 (61.8)	IHC	NR	39 (57.4)	41 (60.3)	26 (38.2)	9
Ying	2007	China	43 (median)	Unselected	I-IV	33 (46.5)	≥1 (56.3)	71	C: 56 (78.9)	N: 49 (69.0)	IHC	Score=1	NR	NR	NR	8
Zhang	2008	China	44.5 (mean)	Invasive ductal cancer	NR	NR	≥1 (40.0)	45	NR	N: 27 (60.0)	IHC	10%	12 (26.7)	32 (71.1)	29 (64.4)	7
Guo	2009	China	32-63	Unselected	I-IV	34 (44.7)	≥1 (55.3)	76	N: 27 (65.8)	NR	IHC	5%	NR	NR	NR	7
Yue	2009	China	25-71	Invasive ductal cancer	I-III	12 (23.5)	≥1 (56.9)	51	C: 56 (78.9)	NR	IHC	10%	19 (37.3)	24 (47.1)	22 (43.1)	8
Qi	2010	China	26-66 (median 46)	Unselected	I-IV	37 (46.3)	≥1 (55.0)	80	N: 52 (65.0)	NR	IHC	5%	NR	NR	NR	6
Rong	2010	China	NR	Unselected	I-III	NR	≥1 (31.3)	64	C>N	NR	IHC	Score=2	44 (68.8)	30 (46.9)	29 (45.3)	6
Li	2011	China	32-67 (50.7±9.4)	Invasive ductal cancer	I-III	NR	≥1 (64.2)	67	42 (62.7)	NR	IHC	Score=3	NR	NR	NR	9
Yang	2011	China	38-72 (51.8±10.1)	Unselected	I-III	NR	≥1 (41.7)	36	C: 10 (58.8)	N: 9 (52.9)	IHC	Score=4	NR	NR	NR	8
Chen	2012	China	23-68	Unselected	I-IV	45 (28.1)	≥1 (65.0)	160	NR	N: 111 (69.4)	IHC	25%	52 (32.5)	65 (40.6)	69 (43.1)	6
Ma	2012	China	29-68 (median 47.2)	Unselected	I-IV	39 (46.4)	≥1 (54.8)	84	NR	N: 59 (70.2)	IHC	Score=1	NR	NR	NR	6
Yang	2012	China	NR	Unselected	I-IV	18 (14.3)	≥1 (66.7)	126	C+N: 98 (77.8)	NR	IHC	Score=1	102 (81.0)	92 (73.0)	72 (57.1)	6
Zhu	2012	China	NR	Invasive ductal cancer	NR	23 (32.9)	≥1 (60.0)	70	NR	N: 44 (62.9)	IHC	10%	NR	36 (51.4)	38 (54.3)	8
Chen	2013	China	32-77 (48.8±10.5)	Unselected	I-III	NR	≥1 (45.7)	140	C: 87 (62.1)	N: 67 (47.9)	IHC	score=2[a]; 25%[b]	41 (29.3)	64 (45.7)	72 (51.4)	9
Zhou	2013	China	29-74 (median 46.7)	NR	I-III	36 (33.3)	≥1 (63.0)	108	C+N: 93 (86.1)	NR	IHC	Score=2	34 (31.5)	58 (53.7)	61 (56.5)	8
Liu	2014	China	29-85 (median 51)	Unselected	I-IV	21 (11.9)	≥1 (58.1)	208	150 (72.1)	91 (43.8)	IHC	Score=6	111 (67.7)	111 (62.0)	107 (59.8)	8
Fang	2014	China	52.3 (mean)	Invasive ductal cancer	I-IV	11 (33.3)	≥1 (60.6)	33	N: 32 (97.0)	NR	IHC	Score=1	NR	NR	NR	6
Guo	2015	China	32-63 (mean 51.6)	Unselected	I-IV	34 (44.7)	≥1 (55.3)	76	50 (65.8)	NR	IHC	5%	NR	NR	NR	6
Wang	2015	China	NR	Invasive cancer	I-IV	104 (27.4)	≥1 (44.1)	379	NR	N: 355 (93.6)	IHC	Score=1	331 (87.3)	283 (74.7)	272 (71.8)	8

Abbreviations: NR, not reported; C, cytoplasm; N, nucleus; IHC, immunohistochemistry; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; [a]STAT3; [b]p-STAT3.

Table 2. Characteristics of included studies for survival analysis

First author	Year	Origin of popula-tion	Histology	Lymph node sta-tus (%)	Number of pa-tients	STAT3+ (%)	p-STAT3+ (%)	Method	Cut-off	Her2+ (%)	Follow-up (months)	Survival analysis	HR statis-tics	HR (95% CI)	P	Quality score
Dolled-Filhart	2003	USA	Unselected	≥1 (0.0)	346	C: 198 (69.2); N: 66 (23.1)	C: 56 (19.6); N: 124 (43.5)	IHC	Score=1	NR	187 (mean)	OS	R	0.35 (0.12-1.03)[a]; 0.43 (0.18-0.99)[b]	0.056[a]; 0.047[b]	6
Yamashita	2006	Japan	Invasive ductal cancer	≥1 (43.8)	517	C: 206 (39.8); N: 7 (1.4)	NR	IHC	10%	107 (30.9)	90 (median)	OS/DFS	SC	1.29 (0.89-1.87)	NR	8
Liu	2007	China	Invasive ductal cancer	≥1 (100.0)	130	NR	83 (63.8)	IHC	Score=3	40 (30.8)	61 (median)	OS/DFS	SC	1.20 (0.76-1.89)	0.332	7
Li	2011	China	Invasive ductal cancer	≥1 (64.2)	67	42 (62.7)	NR	IHC	Score=3	NR	70.6±4.3	OS	R	3.88 (1.12-13.43)	0.013	8
Sato	2011	USA	Unselected	≥1 (39.5)	721	NR	N: 371 (51.5)	IHC	Score=1	NR	150 (total)	OS	R	0.84 (0.62-1.12)	0.238	7
Sonnenblick	2013	Israel	Invasive ductal cancer	≥1 (70.8)	375	NR	N: 134 (35.7)	IHC	10%	36 (12.2)	108 (median)	OS	R	0.48 (0.28-0.84)	0.01	7
Chen	2013	China	Unselected	≥1 (45.7)	140	C: 87 (62.1)	N: 67 (47.9)	IHC	score=2[a]; 25%[b]	41 (29.3)	54 (median)	OS	SC	0.78 (0.21-3.04)[a]; 1.59 (0.75-4.25)[b]	0.029[a]; 0.001[b]	6

Abbreviations: NR, not reported; C, cytoplasm; N, nucleus; IHC, immunohistochemistry; HER2, human epidermal growth factor receptor 2; OS, overall survival; DFS, disease free survival; SC, survival curve; DE, data extrapolated; R, reported; [a]STAT3; [b]p-STAT3.

Table 3. Meta-analysis of STAT3 or p-STAT3 expression and clinicopathological features of breast cancer

	STAT3					p-STAT3					Overall					
				Heterogeneity					Heterogeneity					Heterogeneity		
Clinical features	No. of studies	OR (95% CI)	P	x^2	I^2 (%)	P	No. of studies	OR (95% CI)	P	x^2	I^2 (%)	P	No. of studies	OR (95% CI)	P	x^2	I^2 (%)	P
Age	11	0.92 (0.53-1.60)	0.775	28.11	64.4	0.002	6	1.17 (0.81-1.68)	0.401	2.07	0.0	0.840	14	1.02 (0.73-1.45)	0.892	30.30	47.2	0.016
Tumor size	6	1.06 (0.70-1.60)	0.798	2.51	0.0	0.774	7	1.39 (0.59-3.28)	0.457	19.43	69.1	0.003	10	1.19 (0.78-1.84)	0.420	22.21	46.0	0.035
Grade	8	1.79 (1.13-2.84)	0.014	8.18	14.4	0.317	7	2.29 (1.25-4.21)	0.007	15.96	62.4	0.014	13	2.02 (1.39-2.94)	<0.001	24.58	43.1	0.039
LNM	12	2.99 (1.94-4.62)	<0.001	21.73	49.4	0.027	9	2.94 (2.01-4.30)	<0.001	11.74	31.8	0.163	18	3.00 (2.25-3.99)	<0.001	33.59	40.5	0.029
TNM stage	11	4.26 (2.91-6.23)	<0.001	14.28	29.9	0.161	7	3.78 (2.58-5.55)	<0.001	8.82	32.0	0.184	15	4.03 (3.07-5.27)	<0.001	22.69	25.1	0.160
ER	6	0.76 (0.48-1.19)	0.224	6.30	20.7	0.278	6	0.85 (0.46-1.56)	0.598	13.39	62.7	0.020	11	0.84 (0.57-1.22)	0.357	21.17	48.0	0.032
PR	6	0.78 (0.54-1.13)	0.190	4.29	0.0	0.508	6	1.18 (0.85-1.66)	0.324	4.91	0.0	0.427	11	0.98 (0.77-1.25)	0.870	11.65	5.5	0.391
HER2	5	1.09 (0.71-1.67)	0.686	2.82	0.0	0.589	3	1.25 (0.76-2.06)	0.372	0.06	0.0	0.971	7	1.16 (0.84-1.60)	0.375	3.11	0.0	0.874

Abbreviations: LNM, lymph node metastases; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; OR, odds ratio; CI, confidence interval.

excluded because of duplicate reports, reviews, abstracts, low quality and without sufficient survival data. Eventually, 9 English papers and 15 Chinese papers were selected in this meta-analysis. All of the selection processes were performed by two independent authors (Xiao-Ling Xiao and Kang-Lai Wei).

Study characteristics

Twenty-four studies published from 2003 to 2016 were eligible for meta-analysis and their general features were summarized in **Tables 1** and **2**. The total number of 4,031 patients from America and Asia were all adults with the sample size varied from 33 to 721. STAT3 and p-STAT3 expression were all involved in 5 studies. IHC was the only method to detect the level of STAT3 or p-STAT3 in breast cancer but the cutoff value was different between studies. The association between STAT3 or p-STAT3 expression and clinicopathological parameters was evaluated by 19 studies. Seven studies were related to survival analysis, OS and DFS were reported in 2 researches while other studies reported OS alone as a possible outcome of patients. Mean scores of quality assessment with clinicopathological and prognostic studies were 7.32±1.16 and 7.00±0.82 respectively.

The relationship between STAT3 or p-STAT3 expression and clinicopathological parameters

To analyze the role of STAT3 or p-STAT3 in breast cancer, the association of STAT3 or p-STAT3 expression with clinicopathological parameters was investigated and the result was summarized in **Table 3**. Overall of positive STAT3 and p-STAT3 expression in breast cancer patients revealed significantly associated with the higher histopathological grade (OR=2.02; 95% CI: 1.39-2.94; *P*<0.001) (**Figure 2A**), LNM (OR=3.00; 95% CI: 2.25-3.99; *P*<0.001) (**Figure 3A**) and TNM stage (OR=4.03; 95% CI: 3.07-5.27; *P*<0.001) (**Figure 4A**). The result revealed that high expression of STAT3 was related to higher histopathological grade (OR=1.79; 95% CI: 1.13-2.84; *P*=0.014), LNM (OR=2.99; 95% CI: 1.94-4.62; *P*<0.001) and TNM stage (OR=4.26; 95% CI: 2.91-6.23; *P*<0.001). Similarly, overexpression of p-STAT3 was also associated with grade (OR=2.29; 95% CI: 1.25-4.21; *P*=0.007), LNM (OR=2.94; 95% CI: 2.01-4.30; *P*<0.001) and TNM stage (OR=3.78; 95% CI: 2.58-5.55; *P*<0.001). Furthermore, the cor-relations of positive STAT3 or p-STAT3 expression with remaining clinical features, including age, tumor size, ER, PR and HER2 status, were also investigated. Pooled ORs of positive STAT3 or p-STAT3 expression indicated no obvious correlation with these clinical parameters of breast cancer.

Moreover, sensitivity analysis was performed by removing a single study and the corresponding ORs of STAT3 or p-STAT3 expression and clinicopathological parameters were not significantly altered, indicating the stability of the result. Publication bias of the 19 literatures was predicted through Egger's and Begg's test. Begg's funnel plots of histopathological grade, LNM and TNM stage were shown in **Figures 2B**, **3B** and **4B**. Egger's test *P* value were 0.904, 0.579 and 0.152 respectively, suggesting there was no obvious publication bias in STAT3 or p-STAT3 expression with histopathological grade, LNM and TNM stage.

*Impact of STAT3 or p-STAT3 expression on survival analysis of breast cancer*

The pooled HR and 95 % CI of OS for all 7 studies was 0.90 (95% CI: 0.63-1.28; *P*=0.557), indicating no clear relationship between STAT3 or p-STAT3 expression and prognosis (**Figure 5A**). Subgroup analyses were performed to estimate if origin of population was different from the analysis above. Poor prognosis was observed among breast cancer patients with positive STAT3 or p-STAT3 expression in East Asians (HR=1.32; 95% CI: 1.02-1.72; *P*=0.036) without obvious heterogeneity (x^2=3.85; I^2=0.0%; *P*=0.427), while favorable prognosis in non-East Asians (HR=0.57; 95% CI: 0.37-0.88; *P*=0.012) (**Figure 6**).

Sensitivity analysis was performed and the corresponding pooled HR of STAT3 or p-STAT3 expression and prognosis for all studies was not significantly altered, indicating the stability of our results. Begg's funnel plot was largely symmetric and *P* value of Egger's test was 0.866, suggesting there was no proof of publication bias in STAT3 or p-STAT3 expression and prognosis (**Figure 5B**).

Discussion

Janus kinases (JAKs) activate STAT transcription factors. JAK/STAT and EGFR signaling pathways commonly participate signal regulation in
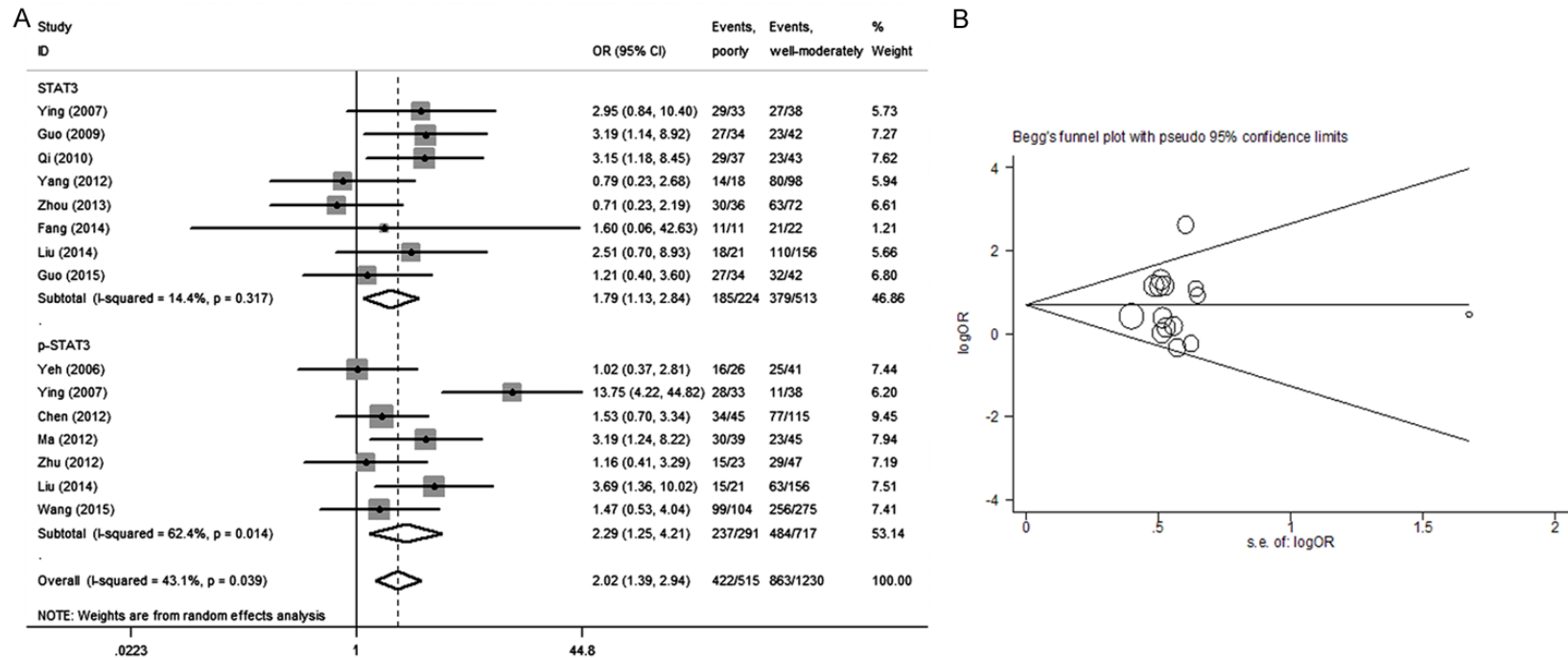
Figure 2. A. Forest plot of pooled odds ratios (OR) comparing positive STAT3 or p-STAT3 expression and histopathological grade in breast cancer patients; B. Begg's funnel plot was designed to visualize a potential publication bias for positive STAT3 or p-STAT3 expression and histopathological grade in breast cancer patients.
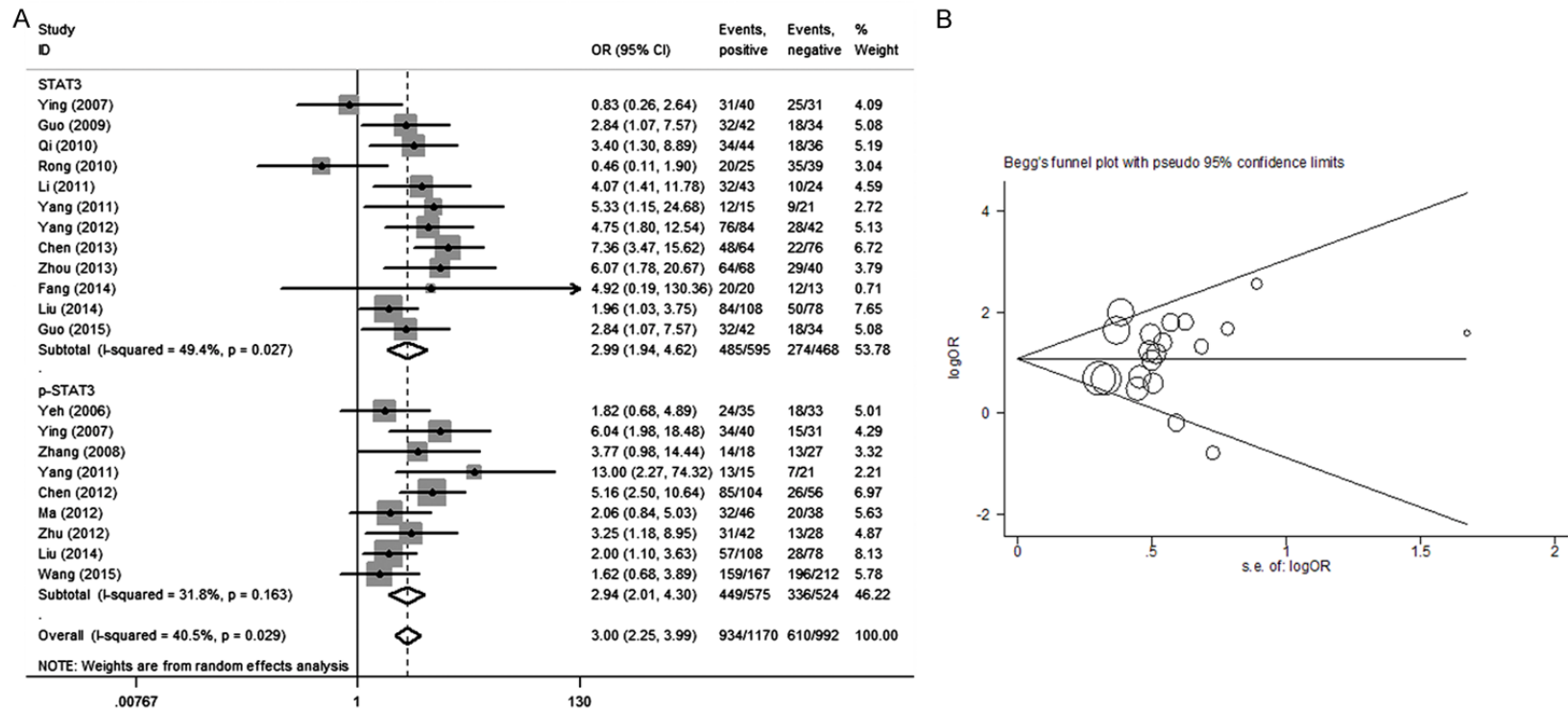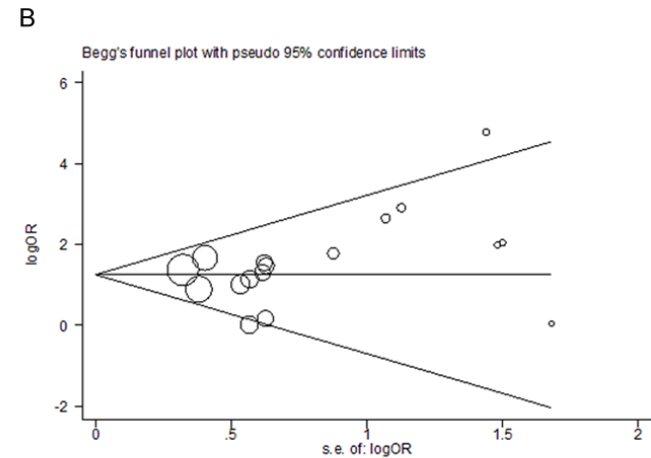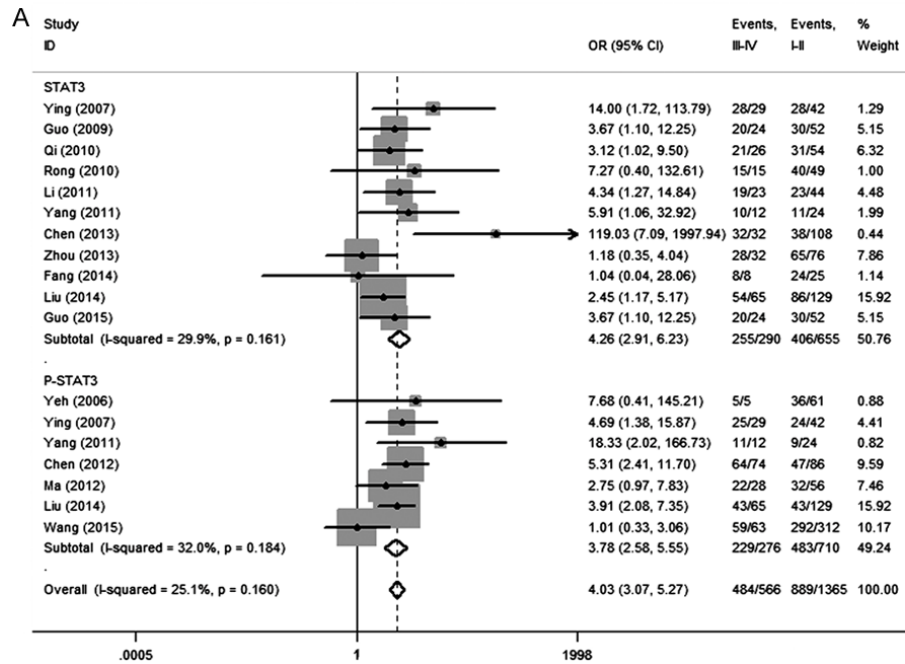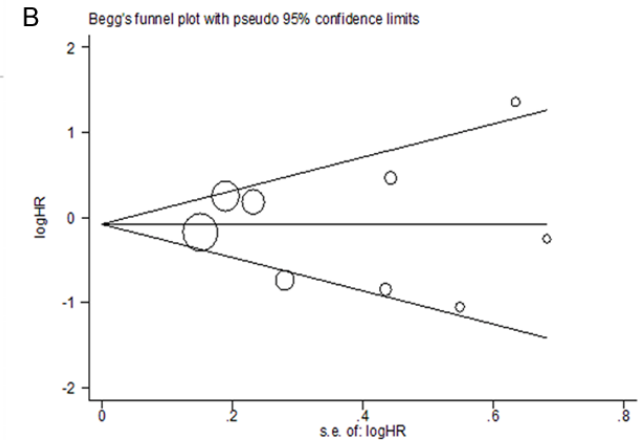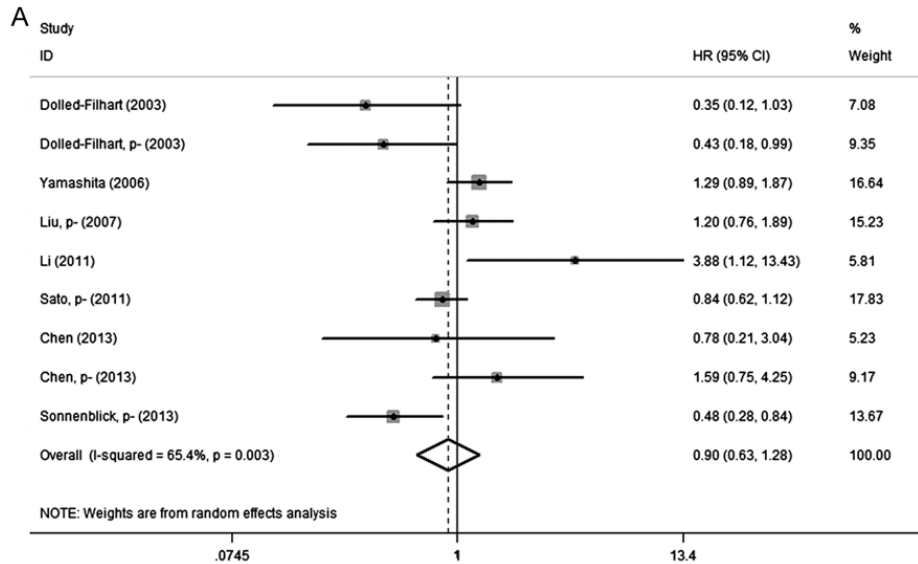
Figure 3. A. Forest plot of pooled odds ratios (OR) comparing positive STAT3 or p-STAT3 expression and lymph node metastases (LNM) in breast cancer patients; B. Begg's funnel plot was designed to visualize a potential publication bias for positive STAT3 or p-STAT3 expression and LNM in breast cancer patients.

Figure 4. A. Forest plot of pooled odds ratios (OR) comparing positive STAT3 or p-STAT3 expression and TNM stage in breast cancer patients; B. Begg's funnel plot was designed to visualize a potential publication bias for positive STAT3 or p-STAT3 expression and TNM stage in breast cancer patients.

Figure 5. A. Forest plot of pooled hazard ratios (HR) for overall survival of positive STAT3 and p-STAT3 expression in breast cancer patients; B. Begg's funnel plot was designed to visualize a potential publication bias for overall survival of positive STAT3 and p-STAT3 expression in breast cancer patients.
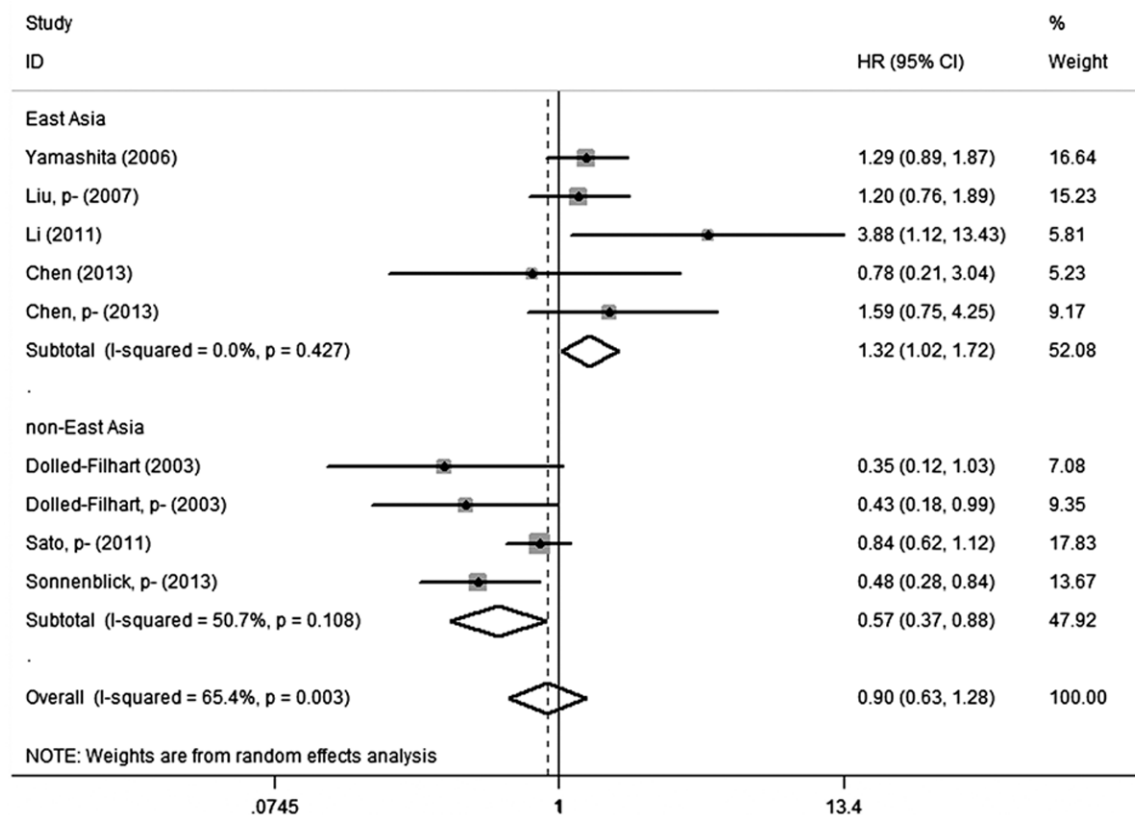
Figure 6. Forest plot of pooled hazard ratios (HR) for overall survival of high STAT3 and p-STAT3 expression, and were stratified by East Asia and non-East Asia.

a variety of cancers and STAT3 is an important downstream protein of these pathways [11]. Activated STAT3 dimerized to p-STAT3, p-STAT conveys messages to nucleus and regulates gene expression in cells. STAT3 and p-STAT3 regulate the transcription of key genes related to cell proliferation, apoptosis, differentiation, immune responses, angiogenesis and metastasis in malignancies [11, 43-45]. In JAK/STAT and EGFR signaling pathways, several downstream proteins such as BCL2 [6], c-Myc [46], VEGF [47] and RTK [48] have been shown to be closely related to progression and prognosis.

In our meta-analysis, all patients were from East Asia in the analysis of STAT3 or p-STAT3 and clinicopathological parameters. Significant association of positive STAT3 and p-STAT3 expression with clinical parameters, including higher histopathological grade, LNM and TNM stage, were displayed in breast cancer. The studies of Liu et al [33] and Guo et al [35] also reported that higher histopathological grade was associated with STAT3 or p-STAT3 overex-

pression in breast cancer. In agreement, recent studies have confirmed high STAT3 or p-STAT3 expression was strongly correlated with LNM [32, 33, 35]. Moreover, STAT3 and p-STAT3 were more frequently expressed in clinical III and IV stages compared with I and II stages [16, 33, 35]. Higher histopathological grade, positive LNM and advanced TNM stage were strong risk factors and significantly related to malignant tumor progression. The results indicated that overexpression of STAT3 and p-STAT3 was associated with deterioration process in breast cancer.

The current results could not make a conclusion for the association of STAT3 or p-STAT3 expression with prognosis. There are several potential reasons for a negative result, including low number of included studies, various subtypes of breast cancer, individual variance of patients, regional diversity of population and various cut-off values for STAT3 and p-STAT3 expression. The association of STAT3 and p-STAT3 with prognosis has obvious regional

difference according to overall analysis of the included literatures. In the survival analysis of STAT3 or p-STAT3 expression in breast cancer patients, 4 studies of East Asia (China [17, 24 31] and Japan [15]) and 3 of non-East Asia (USA [37, 38] and Israel [30]) were included. The study reported by Dolled-Filhart et al [37] and Sonnenblick et al [30] (invasive ductal carcinoma) have demonstrated that overexpression of p-STAT3 was favorable prognostic markers in breast cancer. In contrast, the correlation between positive STAT3 or p-STAT3 expression and poor prognosis in breast cancer was concluded by Liu et al [17] (invasive ductal carcinoma), Li et al [24] (invasive ductal carcinoma) and Chen et al [31]. In the research of Sato et al [38], positive expression of p-STAT3 indicated better overall survival without statistical significance. Yamashita et al [15] showed that STAT3 was unrelated to survival in invasive ductal carcinoma of breast. In the regional subgroup, positive STAT3 and p-STAT3 expression indicated poor prognosis in East Asian population, while favorable prognosis in non-East Asians. The result showed that STAT3 and p-STAT3 expression in different origins may have different outcomes in breast cancer patients, indicating regional differences should be considered when prognostic markers are detected. More qualified large sample researches should be performed to prove the association between STAT3 or p-STAT3 and survival of breast cancer patients.

Breast cancer is a heterogeneous disease, which better diagnostic and prognostic markers for clinical treatment are needed. Inhibitors targeted at EGFR, HER2 and PI3K/AKT/mTOR pathway for breast cancer have been applied in clinical therapies [49-51]. Accumulating studies have reported that STAT3 and p-STAT3 played pivotal role in the development and prognosis of breast cancer [15-38]. In experimental researches, STAT3-targeted inhibitors could suppress tumor progression of breast cancer [52, 53]. However, molecular targeted therapy on STAT3 or p-STAT3 for cancer patients has not been approved to date. This meta-analysis indicates the correlation of clinicopathological features and prognosis with STAT3 or p-STAT3 quantitatively. It is important to find prognostic markers for the treatment and improve the outcome of breast cancer patients.

Our meta-analysis might have several limitations. Firstly, selection bias of the article is likely to exist for positive results were reported in studies, while negative results may not be accepted by journals. Insufficient standard researches were available especially in survival analysis, and only East Asian studies were included in the analysis of STAT3 or p-STAT3 with parameters. Secondly, the primary antibodies selected in IHC and the staining score applied to evaluate STAT3 or p-STAT3 differs among studies, which caused different results and limits their future implementation. Thirdly, in the included studies, the characteristics of patients such as age, lymph node status, treatments and follow-up period also varied widely. Only English and Chinese papers were included. In addition, HRs and their 95% CIs were not reported in some studies and therefore we estimated by calculating from survival data or extracting data from survival curves. However, these methods can not completely eliminate errors.

In conclusion, this meta-analysis indicates STAT3 and p-STAT3 are important predictors of progression of breast cancer. Positive STAT3 and p-STAT3 expression is related to higher histopathological grade, LNM and TNM stage in breast cancer patients of East Asia. Positive STAT3 and p-STAT3 expression might potentially predict prognosis of breast cancer patients in East Asians, while favorable prognosis in non-East Asians. The result might be contributed to guide clinical diagnosis and therapy in different regions. Certainly, further investigations should be concerned to clarify the role of STAT3 or p-STAT3 in breast cancer.

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Disclosure of conflict of interest

None.

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