Prognostic and Clinicopathological Value of Slug Expression in Breast Cancer: A Meta-analysis

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Abstract

1.1 Background

Slug as a member of the epithelial-mesenchymal transition(EMT) leads to the decrease of adhesion between cancer cells and the increase of cell migration. Many studies have been reported the relationship between prognosis and slug expression in patients with breast cancer, yet the results are controversial. Therefore, it is necessary to conclude the relationship between slug and breast cancer by a meta-analysis.

1.2 Methods

We looked for studies on breast cancer and slug via PubMed, Scopus and Web of Science .A total of 1458 patients from the final eight studies were included in this meta-analysis. Overall survival (OS) and Disease-free survival (DFS) were the primary endpoints. Pooled hazard ratio (HR), pooled odds ratio (OR), and 95% confidence interval (CI) were calculated to assess the association between slug, prognosis, and clinicopathological parameters (age, tumor size, histological grade, axillary lymph nodes status(LN),TNM stage, ER(estrogen receptor) status, PR(progesterone receptor) status, HER-2(human epidermal growth factor receptor 2)status). Data analysis was carried out by using STATA version 14.0. (Stata Corporation, TX, USA)software.

1.3 Results

This meta-analysis included 1458 patients from eight studies. The final results show that high slug expression leads to poor OS [pooled HR = 2.21;95%CI=1.47-3.33;p<0.001) and DFS [pooled HR = 2.03;95%CI=1.26-3.28

; P=0.004) in breast cancer. In terms of clinicopathological parameters, the results show that breast cancer patients with high slug expression have higher TNM stage(I-II/III-IV; pooled OR=0.42;95%CI=0.25-0.70;P=0.001), more prone to axillary lymph node metastasis(N+/N0;pooled OR =2.16; 95%CI =1.31-3.56; P=0.003) and more severe ER deficiency (positive /negative; pooled OR=0.67;95%

CI=0.45-0.99; P=0.042).However, slug is not related to age, histological grade, tumor size, PR status and Her-2 status.

1.4 Conclusion

This meta-analysis results show that high slug expression in breast cancer is associated with poor OS, DFS and axillary lymph node status, TNM stage and ER status, but not related to age, histological grade, tumor size, PR status and Her-2 status.

2. Introduction

Breast cancer is the most common cancer among women worldwide, which is the leading cause of death among women aged 20-50 [1].In the 2018 global cancer statistics, breast cancer accounted for about 11.6% of all cancer cases, tied with lung cancer. There were 0.63 million breast cancer deaths a year [2]. The annual incidence rate is on the rise [3].Which suggests that breast cancer has a significant impact on women's health, so it is important to reduce the incidence and improve the prognosis. There are many common clinicopathological parameters include age, axillary lymph nodes status,tumor size, histological grade, TNM stage, PR status, ER status, HER-2 status[4].But none of them could accurately predict the prognosis[5].In the context of precision medicine, we need to identify new prognostic biomarkers in breast cancer, to classify groups of patients with poor clinical outcomes who may benefit from new individualized treatments[6].

Slug (also termed snail2) is a C₂H₂ zinc-finger transcriptional repressor belonging to the three-member family of snail proteins (Snail, Slug, and Smuc), which mediates sequence-specific interactions with DNA [7], which has many biological functions, such as cell migration, cell invasion, cell cycle regulation, and stem cell characteristics in tumor cells[8]. What's more, slug is highly expressed in a variety of cancer cells ,including lung cancer, breast cancer, ovarian cancer, esophageal cancer, pancreatic cancer and colorectal cancer etc [9] . High expression of slug in breast cancer is associated with higher TNM stage and increased metastatic potential [10, 11].In addition, many studies have found that high expression of slug in breast cancer cells is associated with multiple drug resistance, including resistance to chemotherapy and endocrine therapy [12, 13].This makes slug a potential predictor of poor prognosis in breast cancer

Many studies have explored the role of slug in the clinicopathological parameters and prognosis of breast cancer, but the results were inconsistent. It has been reported by Wan et al that high expression of slug in breast cancer was correlated with poor survival [14]. However, it is worth noting that Imani et al[15] and Huang et al[16] found that the expression of slug in breast cancer was not related to the prognosis [17]. In the face of controversy, it is necessary to do a meta-analysis to determine the relationship between high slug expression in breast cancer and prognosis and clinicopathological parameters (age, tumor size, lymph node metastasis, histological grade, TNM stage, ER, PR, HER-2).

3. Methods

3.1 Publication search

Search PubMed, Scopus and Web of Science for articles (for dates up to December 01, 2020) with the Keyword: "SLUG" or "SNAIL2" or "SNAI2", "breast cancer" or "breast neoplasm" or "breast tumor". In addition, a further manual search of the reference list of eligible studies was conducted to identify additional relevant studies.

3.2 Study selection

Studies that meet the following criteria may be included: (1) All studies had prognostic or clinicopathological results. (2) Slug expression in breast cancer cells was detected by immunohistochemistry (IHC). (3) A prognostic indicator of HR is extracted directly or extrapolated indirectly. Summaries of meetings, letters, reviews, meta-analyses, animal model studies, and studies that did not provide sufficient data were excluded.

3.3 Data extraction

The data of the papers were extracted by two researchers (Zhang and Yang). When disagreements arose, they resolved through discussion. Data extraction includes: Authors, country, year of publication, including number of patients, mean age (year) and median follow-up time (months), survival data, and clinicopathological parameters. In addition, we also extracted the slug antibody dilution, location and cut-off value. For prognosis of HR we extract directly from the paper, if the HR can't be extracted directly, we choose to extract from Kaplan-Meier Curve via Engauge Digitizer Version 4.1 ([http:// markum mitc hell. github. io/engauge-digi tizer/](http://markum.mitc.hell.github.io/engauge-digitizer/)). We evaluated the quality of articles using the Newcastle Ottawa Scale (Nos) [18], which has a maximum score of 9, and we will discard articles with a score of 5 or less.

3.4 Statistical analysis

We used STATA version 14.0 (Stata Corporation, TX, USA) software to analyze data, the clinicopathological parameters were eventually combined as OR, and the prognostic indicators were finally combined as HR. If we can't extract HR directly, according to Tierney et al, Kaplan-Meier curve was used to extract the data [19]. P value ≤ 0.05 was considered statistically significant. Cochran Q test and I^2 statistics were used to measure heterogeneity, P value < 0.1 and I^2 value $> 50\%$ represented strong heterogeneity [20]. At this point the random model was used. Otherwise, the fixed-effects model was used [21]. Sensitivity analysis was used to check data stability, The Egger's test was used to detect publication bias, with P value ≤ 0.05 indicating publication bias.

4. Results

4.1 Search results

A total of 1,186 articles were retrieved using the search strategy (Fig. 1). 649 records saved after duplicates removed. Next, 596 were excluded after screening of the titles and abstracts. By reading the full text of 53 articles, 43 articles were found to have no available outcome indicators, and 2 articles were bioinformatics analysis. A total of 1458 patients were included in the final eight studies [10, 14, 22-27]. The detailed parameters of these articles are listed in Table 1 and Table 2. The quality of all articles was listed (Supplementary Table 1).

4.2 Correlation between slug expression and prognosis

A total of 5 articles provide OS related data. Due to the less heterogeneity ($I^2=4.0\%$) We used the fixed-effect model to pool HR—the combined results show that high expression of slug associated with poor OS—pooled HR= 2.21;95%CI=1.47-3.33;p<0.001) (Fig.2a). A total of 5 studies provided DFS-related data because there was less heterogeneity ($I^2=26.8\%$) We used the fixed-effect model to pool HR—the combined results show that high expression of slug associated with poor DFS—pooled HR = 2.03;95%CI=1.26-3.28;P=0.004) (Fig.2b).

4.3 Correlation between slug expression and clinicopathological parameters

A total of 7 articles provide clinicopathological related data, the specific characteristics were showed in Table 2. The clinicopathological parameters pooled OR values were showed in Table 3. The results show that breast cancer patients with high slug expression have higher TNM stage (I-II/III-IV; pooled OR=0.42; 95%CI=0.25-0.70; P=0.001) (Supplementary Figure 1), more prone to axillary lymph node metastasis (N+/N0; pooled OR =2.16; 95%CI =1.31-3.56; P=0.003) (Supplementary Figure 2) and more severe ER deficiency (positive /negative; pooled OR=0.62; 95%CI=0.41-0.93; P=0.021) (Supplementary Figure 3). However, this study shows that slug expression is not associated with patient age (≤ 50 / >50 ; pooled OR =1.15; 95%CI=0.80-1.64 ;P=0.455) (Supplementary Figure 4) histological grade (I-II/III; pooled OR =0.58;95%CI=0.30-1.12;P=0.104) (Supplementary Figure 5), tumor size (≤ 2 cm / >2 cm; pooled OR =0.90;95%CI=0.49-1.65; P=0.737) (Supplementary Figure 6), PR status (positive/negative; pooled OR= 0.84; 95%CI =0.38-1.85; P=0.661) (Supplementary Figure 7) ,HER-2 status (positive/negative ; pooled OR=0.70;95%CI=0.47-1.06;P=0.089) (Supplementary Figure 8).

4.4 Sensitivity analysis and publication bias

Our analysis of publication bias using Egger's test correlation test revealed no bias for OS (P =0.751) (Fig.3a) DFS (P = 0.596) (Fig.3b) and all clinicopathological parameters. In addition, the sensitivity analysis showed that the results were reliable for OS—DFS and all clinicopathological parameters.

5. Discussion

Many studies have explored the role of slug in the prognosis and clinicopathological parameters of breast cancer, but the results were inconsistent. Therefore, we determined the relationship between high slug expression in breast cancer and prognosis and clinicopathological parameters by this meta-analysis. Our meta-analysis shows that high expression of slug in breast cancer is associated with worse OS—pooled HR= 2.21;95%CI=1.47-3.33;p<0.001—and DFS(pooled HR=2.03; 95%CI = 1.26 -3.28;P=0.004). To further investigate the role of slug in breast cancer—we analyzed the relationship between slug and clinicopathological parameters of breast cancer—the results show that high expression of slug in breast cancer have higher TNM stage(I-II/III-IV; pooled OR=0.42;95%CI=0.25-0.70;P=

0.001),more prone to axillary lymph node metastasis(N+/N0; pooled OR=2.16; 95%CI = 1.31-3.56;P=0.003) and more severe ER deficiency (positive / negative; pooled OR =0.62; 95% CI= 0.41 -0.93;P=0.021)—and it is worth noting that the relationship between high expression of slug and ER status has been demonstrated in many studies[28, 29].

Slug is associated with prognosis in a variety of cancer cells. Liu et al.[30] and Song et al.[31]found that slug is expressed more in lung cancer cells than in normal lung tissue, and high expression of slug in lung tumor cells is associated with poor survival and more aggressive clinicopathological parameters. Chang et al. [32] and Gu et al.[33] found that slug is highly correlated with the invasiveness and drug resistance of ovarian cancer cells. Toiyamo et al. [34] found that slug expression was significantly increased in colorectal cancer with high T stage, liver metastasis and lymph node metastasis, it may be a potential prognostic marker for colorectal cancer. The relationship between slug and solid tumor was analyzed in a previous study [16] , interestingly ,it found that slug is associated with a poor prognosis in lung cancer—head and neck carcinoma—urinary cancer—gastrointestinal tract carcinoma, but not in breast cancer.

Metastasis of tumor cells and the drug resistance to anti-tumor therapy are the main causes of poor prognosis in cancer patients[35]. Pan et al.[36]and Shao et al.[37]demonstrated that slug is an important factor in promoting the metastasis of breast cancer cells and may be an important marker of metastasis potential. Slug, as a member of EMT, was initially recognized for its

involvement in the EMT project. During the progression of breast cancer, cells and cell adhesion are lost in the EMT process, resulting in migration and invasion [38, 39]. There are many molecular mechanisms that slug promotes the metastasis of cancer underlying EMT. Liu et al [40] found that slug represses the expression of miR-200b and miR-1, and the inhibition of miR-200b and miR-1 promote EMT and tumor cell invasiveness. Fazilaty et al [41] found that slug can induce TNC and Postn by signal cascade, thus promoting the invasiveness of tumor cells. What's more, Lamouille et al. [42] found that the high expression of slug decrease the expression of epithelial genes and activate the expression of mesenchymal genes, which promotes the metastasis of the tumor cells.

High expression of slug can lead to multiple drug resistance [12, 13]. Slug has recently been found to play an important role in tamoxifen resistance of breast cancer [29, 43]. The expression of slug prevented the killing effect of tamoxifen on ER(+) breast cancer cells [44]. Slug has been shown to induce endocrine therapy resistance in breast cancer cells by altering the cell survival signaling pathways, leading to worse DFS [45]. Musgrove et Al. found that the loss of ER expression due to high expression of slug is the main cause of drug resistance in tamoxifen [46]. Some studies suggested that slug induces tamoxifen resistance by increasing EGFR expression and Erk phosphorylation [47]. what's worse, Li et al. [48] found that slug can induce chemotherapy resistance in cancer cells via PI3K/Akt/GSK3b pathway. These studies confirm that slug leads to poor prognosis in breast cancer, consistent with our Meta-analysis results.

Our meta-analysis shows that slug is a key biomarker for predicting prognosis in breast cancer patients, which is the main finding of this study. No heterogeneity or publication bias was found in this meta-analysis, and sensitivity analysis suggested that our results were reliable. There were limitations in our meta-analysis. First, there was no uniform scoring criterion to define the cut-off of the high expression of slug. Second, there were differences in the type and dilution of immunohistochemical antibodies. Third, HR reliability of some prognostic parameters obtained from Kaplan-Meier curve was poor. Fourth, even though we screened through 1,186 articles, we ended up with only eight available studies, and only one of them from a non-Asian population, so more studies were needed in non-Asians in the future.

6. Conclusion

In conclusion, this meta-analysis showed a relationship between slug and poor prognosis in breast cancer. We think slug could be used not only as a biomarker for predicting prognosis, but perhaps as a therapeutic tool in the future. All of which suggests we should do some more research on slug.

Declarations

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Availability of data and materials

The data we generated or analyzed in this study are all included in this manuscript and its additional files.

Authors' contributions

Conceptualization: Xiao Xie, Xue Wei

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Writing-review & editing : Ming Yang

Ethics approval and consent to participate

An ethics statement was not required for this study, no human or animal subjects were used.

Ethics approval and consent to participate

Not applicable.

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Tables

Table 1

haracteristics of studies included.

Author	Year	Country	MA	Stage	NP	MF	Cut-off	Dilution	Location	Positive	HR(e)	NOS
			(Year)				(month)					
									Rate			
Wu	2019	China	55.3	I-III	137	NR	Scores $\geq$ 3	1:50	nuclear	24.10%	curve	8
Ito	2015	Japan	54	IV	47	61	$\geq$ 5%	1:500	nuclear	40%	R	9
Gu	2019	China	NR	I-IV	108	88.5	Scores $\geq$ 4	1:600	cytoplasm	46.30%	curve	9
Wan	2017	China	NR	NR	314	240	Scores $\geq$ 4	NR	nuclear	75.90%	R	9
Prasad	2009	India	56	NR	98	NR	$\geq$ 10%	1:50	nuclear	34%	R	7
Cao	2015	China	NR	I-IV	200	NR	$\geq$ 10%	1:50	nuclear	42%	R	7
Liu	2013	China	52	I-IV	441	NR	Scores $\geq$ 4	NR	NR	39.50%	R	8
Wu	2012	USA	NR	I-III	113	NR	NR	1:100	nuclear	44%	R	6
NR = not reported; MA=mean age; NP=No. of Patients; MF=median follow-up; e=estimate,R=Reported												

Table 2

Data extracted from studies included.

Study	Slug	Age	Tumor size	H grade	LN	TNM stage	ER	PR	HER-2	HR
		≤50/≥50	≤2cm/≥2cm	G1-G2/G3	P/N	II/III-IV	P/N	P/N	P/N	
Wu 2019	High	17/16	12/21	24/9	17/16	NR	NR	NR	NR	OS/DFS
	low	44/61	33/72	81/24	25/80					
Ito 2015	High	8/15	5/18	NR	NR	NR	17/6	NR	NR	DFS
	low	7/17	8/16				16/8			
Gu 2019	High	NR	40/18	44/14	21/37	47/11	44/14	29/29	17/40	OS/DFS
	low		32/18	41/9	20/30	43/7	43/7	35/15	21/23	
Wan 2017	High	55/77	71/61	90/42	62/70	NR	76/56	50/82	47/85	OS/DFS
	low	19/23	21/21	34/8	11/31		26/16	10/ 32	19/23	
Prasad 2009	High	22/11	NR	23/10	43/22	NR	NR	NR	NR	NR
	low	40/25		33/32	19/14					
Cao 2015	High	NR	NR	43/29	39/33	57/15	31/41	34/38	52/20	NR
	low			93/25	50/78	114/14	75/53	71/57	94/34	
Liu 2013	High	45/33	45/33	21/57	57/21	43/35	NR	NR	NR	OS/DFS
	low	30/25	39/6	30/15	15/30	37/8				
Wu 2012	High	NR	NR	NR	NR	NR	NR	NR	NR	OS
	low									
H=Histological;G=Grade;P=Positive;N=Negative;NR=no report; LN=lymph nodes;										

Table 3

Relationship of slug and clinicopathological parameters of breast cancer

Features	OR (95% CI)	P Value	χ^2	Model
Age(≤50/≥50)	1.15(0.80,1.64)	0.455	0.00%	fixed
Histological grade (G1 + G2/G3)	0.58(0.30,1.12)	0.104	73.20%	random
Tumor size(≤2cm/≥2cm)	0.91(0.64,1.28)	0.577	63.80%	random
LN(N+/N0)	2.16(1.31,1.56)	0.003	61.80%	random
TNM (I + II/III + IV)	0.42(0.25,0.70)	0.001	0.60%	fixed
ER status(positive/negetive)	0.67(0.45,0.99)	0.042	0.00%	fixed
PR status(positive/negetive)	0.84(0.38,1.85)	0.661	72.90%	random
HER-2 status(positive/negetive)	0.70(0.47,1.06)	0.089	0.00%	fixed

Figures

Figure 1

Flow chart of study selection process.

Figure 2

Forest plot depicting association between slug expression and OS(2a) and DFS(2b) in breast cancer.

Figure 3

Funnel plot of Eegger's test for publication bias: OS (3a) and DFS 3(b)

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