Prognostic Role of Epithelial Mesenchymal Transition Transcription Factors in Carcinoma- A Systematic Review and Meta-Analysis

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Abstract

Background: We conducted the present meta-analysis to delineate the prognostic significance of epithelial to mesenchymal transition- transcription factors in carcinoma patients.

Method: For the determination of the pooled hazard ratio (HR) values based on the fixed- effects model, in this retrospective study, we employed comprehensive meta-analysis software.

Results: This retrospective analysis identified the expression patterns of 6645 patients in 36 studies. Expression of TWIST1 correlated with the least prognosis rate (HR= 2.129, 95% CI= 1.373 - 3.302) as compared to SNAIL1 (HR= 1.804, 95% CI= 1.151 - 2.827), SLUG (HR= 1.724, 95% CI= 0.992 - 2.997) and ZEB1 (HR= 1.590, 95% CI= 1.358 - 1.861).

Conclusion: These findings suggested the implication of TWIST1 as an effective biomarker for an early tumor diagnosis and therapy for metastasis.

Keywords: Metastasis, EMT-TFs, Fixed effects model, Cancer prognosis, Biomarker

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Introduction

Cancer is the second leading cause of death, accounting for about 9.6 million deaths around the world according to the reports by World Health Organization 2018.[1] This disease begins as an abnormal growth of cells in a primary site and later spreads to distant organs through circulation leading to metastasis. Most therapies fail once cancer proceeds to metastasis stage. It has been estimated that 90% of cancer death occurs due to metastasis.[2] Thus, it becomes imperative to understand the mechanism of metastasis and to identify novel targets for efficient cancer treatment.

Epithelial to mesenchymal transition (EMT), the first step of metastatic cascade, is characterized by low expression of cell adhesion

receptors, such as E-cadherin and tight junction protein-1, that are involved in cell-cell attachment and higher expression of adhesion receptors, like N-cadherin and vimentin, which help in cellular motility.[3] Both intrinsic and extrinsic stimuli activate EMT to various signaling pathways, thereby, inducing transcription factors, including SNAIL1, ZEB1, SLUG, and TWIST1 families for establishing the mesenchymal phenotype of the tumor cell. These transcription factors lead to cellular plasticity when differentially expressed, and ultimately result in tumor initiation, metastatic spread, and chemo-resistance.[4] TWIST1 belongs to basic helix- loop helix (bHLH) family, SNAIL1 and SLUG are zinc-finger transcriptional repressors, and ZEB1 comes under double zinc-finger E-box binding homeobox. These transcriptional factors function by directly binding to the E-box element of E-cadherin promoter and repress the expression of E- cadherin.[5]

Owing to the strong influence of EMT - transcription factors (EMT-TFs) on tumor progression, they are widely utilized to understand the prognosis of tumor and cancer treatment. There are several reports suggesting the role of EMT-TFs for poor prognosis in a variety of carcinomas. However, controversy exists due to the variation in the study group and the methods employed to study them. The present meta-analysis study aimed to investigate the prognostic significance of EMT-TFs (TWIST1, ZEB1, SNAIL1, and SLUG) expression in various cancers. This might help the identification of an effective biomarker for early diagnosis and therapy for metastasis.

Methods

Search strategy and selection of studies

A comprehensive systemic search in PubMed was carried out to retrieve the literatures published in English. Only the papers published between 2010 and 2017 were included in the study. The search was conducted by deriving the heading from the question of search like: "TWIST1",
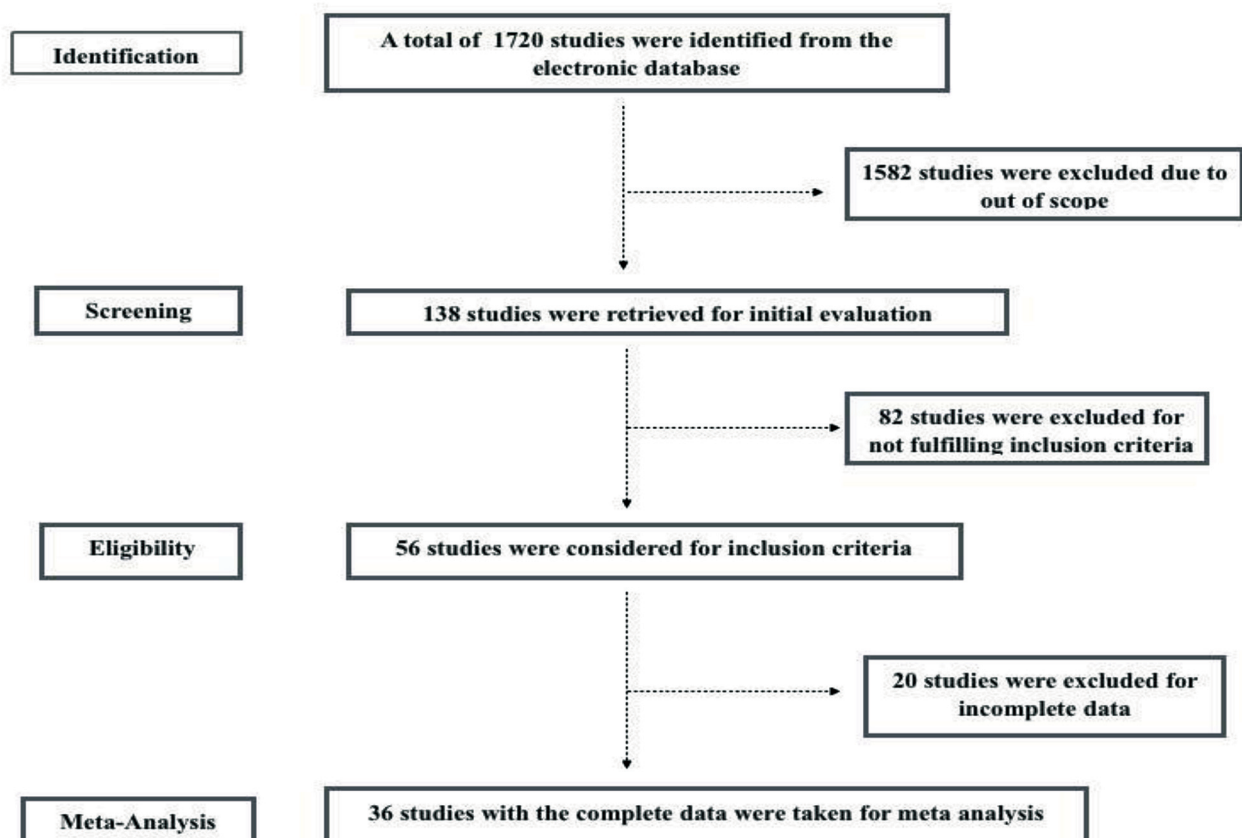

Figure 1. This flow diagram represents the strategy for the selection of studies for the meta-analysis.

"TWIST1 and colon cancer", "TWIST1 and cancer prognosis", or "TWIST1 and colon cancer metastasis". Similar searches were done for different types of carcinoma and the search was also performed with combinations of ZEB1, SNAIL1, and SLUG. Alternate spelling and synonyms were searched with Boolean "OR" and "AND" term. The present study complies with the PRISMA guidelines. Two researchers independently reviewed the identified articles to determine their eligibility for the systematic review. All disagreements between the researchers were resolved and a consensus was reached.
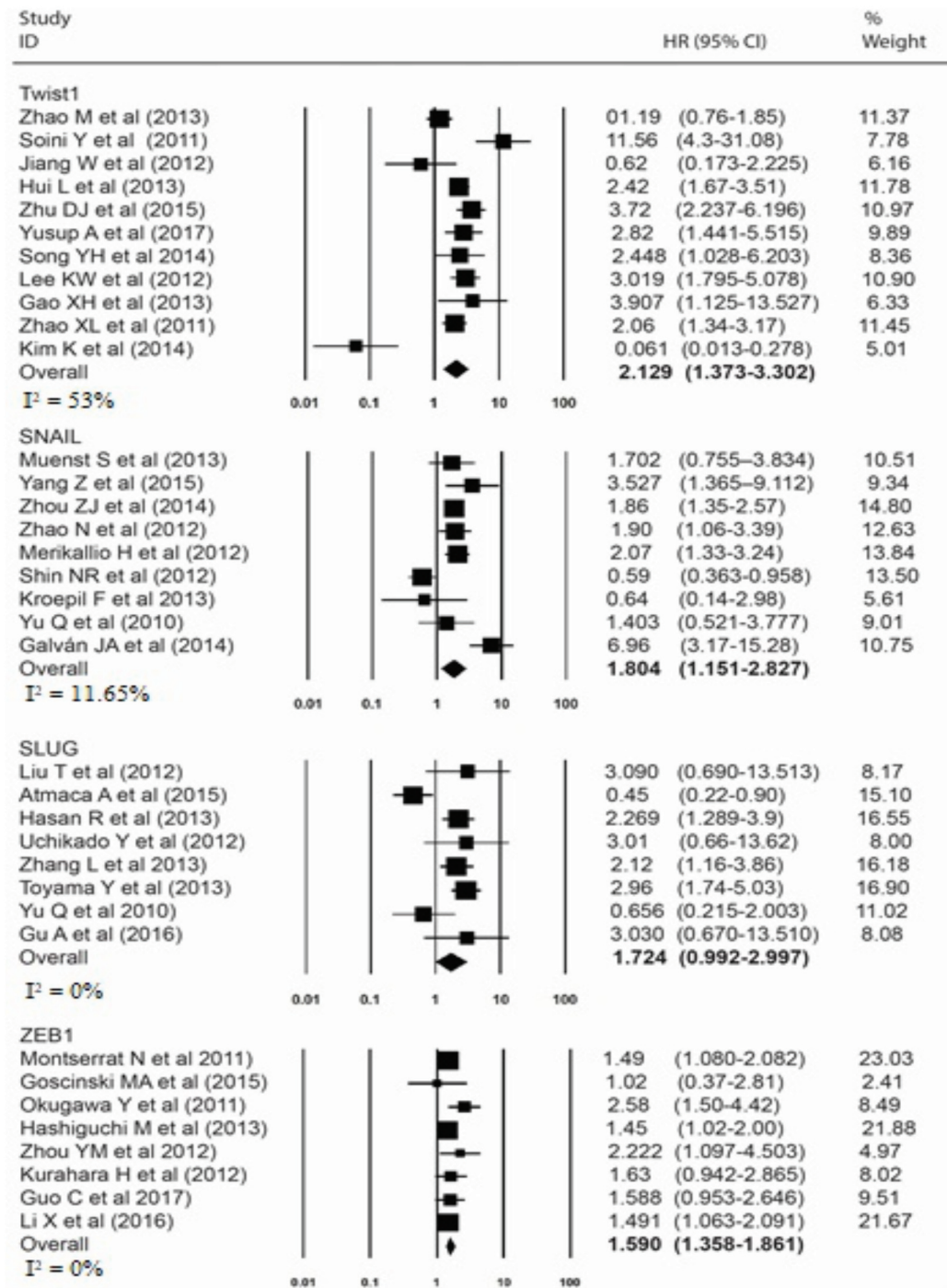

Figure 2. This figure shows the result of the meta-analysis concerning the relationship between EMT-TF's expression and prognosis of carcinoma.

Exclusion and inclusion criteria

The study was met with certain criteria to be eligible for the meta-analysis as follows: (i) Expression of the EMT-TFs (TWIST1, ZEB1, SNAIL1, and SLUG) evaluated through quantitative real time- polymerase chain reaction (RT-PCR) and immunohistochemical (IHC) analysis ; (ii) studies providing survival data; (iii) articles published in English.

The exclusion criteria for this study included: (i) reviews and publications not available in English; (ii) experiments performed on cell line and animal models; (iii) studies without expression data of the EMT-TFs; (iv) studies assessing the expression of the EMT-TFs by microarray. The retrieved articles were manually screened to ensure the sensitivity.

Data collection and study assessment

We analyzed all the articles according to PICO principle. The following data were collected from the eligible studies: primarily, the author's name, year of publication, geographical location, number of patient cases, tumor site, detection methods, positive expression rates of EMT-TFs, outcome, follow-up period, Newcastle- Ottawa quality assessment scale (NOS), P-values for overall survival (OS), and hazard ratios (HRs) with their corresponding 95% confidence interval (CI). The quality of each study was assessed with NOS score. The studies with a score of six or more were considered to be of high quality. The entry was considered as not reported (NR), when none of the required information was reported in the original study.

Statistical analysis

We performed meta-analysis using the Comprehensive Meta-analysis Software (version 3.3.070; Biostat, Inc., USA). The pooled HR value was determined individually for TWIST1, SNAIL1, SLUG, and ZEB1 in order to determine their specific roles in the prognosis of carcinoma. A pooled HR value > 1 denotes a poor prognosis of carcinoma and P- value < 0.05 indicates their statistical significance. The statistical heterogeneity was investigated utilizing forest plot and I^2 test value. I^2 value of greater than 50% indicated the statistical significance of heterogeneity. HR was calculated based on the fixed-effects model, as the heterogeneity in the samples was not statistically significant. We employed Begg's Funnel Plot and Egger's Regression test to detect the presence of publication bias. A value of $P >$ 0.05 indicates the absence of potential publication bias.
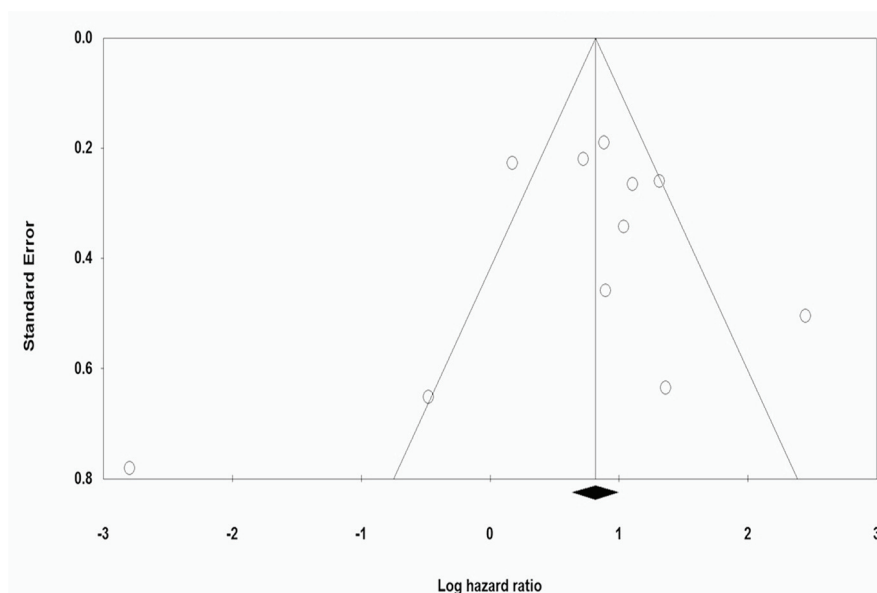

Figure 3. Funnel plot analysis to detect publication bias for the expression of TWIST1. The horizontal axes represent the 95% confidence limits of logarithmic hazard ratio and vertical axes represent standard error of logarithmic hazard ratio.

Results

Literature search

The literature search resulted in 1720 relevant studies. Among these, 138 studies were considered for the initial evaluation. Subsequently, 82 studies were excluded based on the exclusion criteria, and the remaining 56 studies were selected for the inclusion criteria. After screening, 20 studies were precluded due to insufficient data. Finally, 36 studies with complete data were chosen for meta-analysis. A flow diagram representing the selection of studies for the meta-analysis is shown in figure 1.

Description of the studies

We identified 36 studies that satisfied the inclusion criteria for the assessment of the EMT-TFs expression through meta-analysis. The data were distributed into 11 studies (30.55%) reporting 1490 cases (22.42%) for TWIST1,[6-16] 9 studies (25%) reporting 2834 cases (42.64%) for SNAIL1,[17-25] 8 studies (22.22%) reporting 1286 cases (19.35%) for SLUG,[26-33] and 8 studies (22.22%) reporting 1035 cases (15.57%) for ZEB1.[34-41] A total of 6645 patients were included and the median trial sample size was 185 patients. The median follow-up period for the 19 reported studies was 50 (18–115) months.

The variables from the 36 studies are listed in table1. Cancer types varied across studies, with six cases each on breast and liver cancer, five cases evaluated colorectal carcinoma, four cases each reported on lung and gastric cancer, three cases on esophageal and ovarian cancer, and one each evaluated pancreatic and pulmonary NET (Neuro Endocrine Tumour) carcinoma. The information regarding HRs and 95% CI were directly retrieved from the studies. The majority of these studies were conducted in Asia (28 studies, 77.77%) and only a few studies in Europe (7 studies, 19.44%) and America (1 study, 2.77%). The methods applied for detecting the expression of EMT-TFs in the tissue samples of patients were IHC and qRT-PCR.

Meta-analysis results

The association between the EMT-TFs expression and the OS of cancer patients is represented in figure 2. The analysis of 36 studies concluded that the expression of TWIST1, SNAIL1, SLUG, and ZEB1 profoundly affected the prognosis of carcinoma. The highest impact on the prognosis of carcinoma belonged to TWIST1 (HR= 2.129, 95% CI= 1.373 to 3.302), followed by SNAIL1 (HR= 1.804, 95% CI= 1.151 to 2.827), SLUG (HR= 1.724, 95% CI= 0.992 to 2.997), and ZEB1 (HR= 1.590, 95% CI= 1.358 to 1.861). Since the heterogeneity for each specific
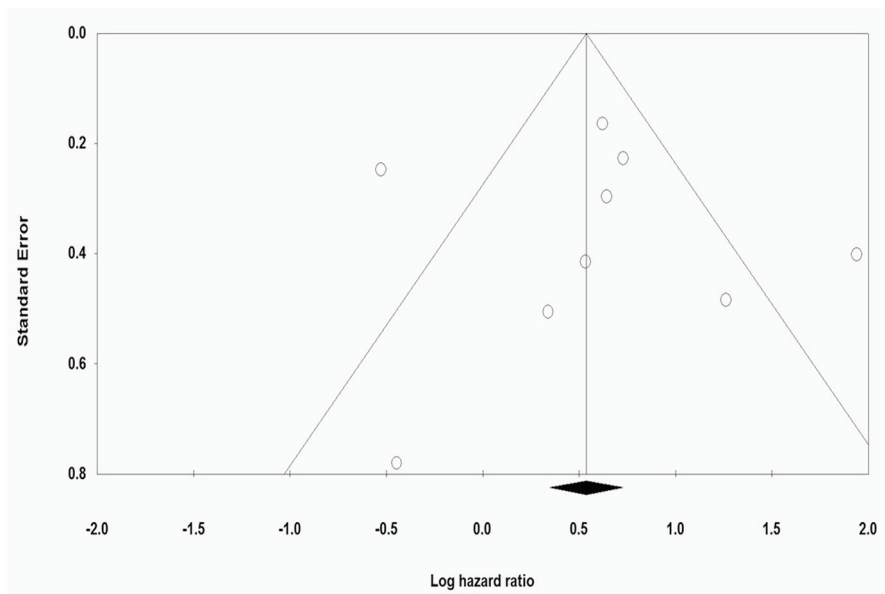

Figure 4. Funnel plot analysis to detect publication bias for the expression of SNAIL1. The horizontal axes represent the 95% confidence limits of logarithmic hazard ratio and vertical axes represent standard error of logarithmic hazard ratio.

analysis was low, (TWIST1: I2 =53%, SNAIL: I2 = 11.65%, SLUG: I2= 0%, ZEB1: I2= 0%) we used the fixed-effects model to determine the HR values.

Publication bias analysis

According to figures 3-6, there is no statistical evidence for publication bias based on the shape of the Funnel Plot and Egger's Regression test (TWIST1: *P*-value= 0.310, SNAIL1: *P*-value= 0.400, SLUG: *P*- value= 0.420, ZEB1: *P*-value= 0.241). The lack of publication bias in the overall result of this meta-analysis indicated that the obtained results were from reliable data.

Discussion

Despite the considerable progress in the cancer diagnosis and newer therapies, there is an anticipated death of 13 million cases by 2030 due to cancer worldwide. Metastasis is one of the major reasons for the failure in cancer treatment. It is the process by which neighboring tissues are invaded from the primary oncogenic site and form new tumor lesions in distant organ site via blood and lymphatic circulation. EMT is believed to play a vital role in the cancer metastatic development and cancer relapse. EMT is characterized by the conversion of E- cadherin to N- cadherin, which results in the loss of ability of the tumor cell to attach to one another.[4]

There are numerous intrinsic and extrinsic stimuli influencing various signaling pathways, like wingless-related integration site (Wnt), Ras, serine/threonine specific protein kinase (Akt), mitogen-activated protein kinase (MAPK), and signal transducer and activator of transcription 3 (STAT3) pathways.[5] Intrinsic factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), hypoxia inducing factor-2 alpha (HIF-2α), metalloproteinases (MMPs), transforming growth factor-beta (TGF-β), estrogen receptor- alpha (ER-α) were shown in many previous studies to activate EMT.[4] Among the extrinsic factors, hypoxia, tumor microenvironment, angiogenesis, and cancer stem cell markers promote EMT. These factors stimulate transcription factors such as TWIST1, SNAIL1, SLUG, and ZEB1 for the transcriptional repression of E-cadherin. This is compelling evidence regarding the role of epigenetics in EMT. The TWIST1/Mi2/NuRD (nucleosome remodeling) protein complex represses ER-α expression by the subsequent hyper methylation and hypo acetylation of the E-cadherin resulting in breast cancer metastasis. Moreover, there exists a long

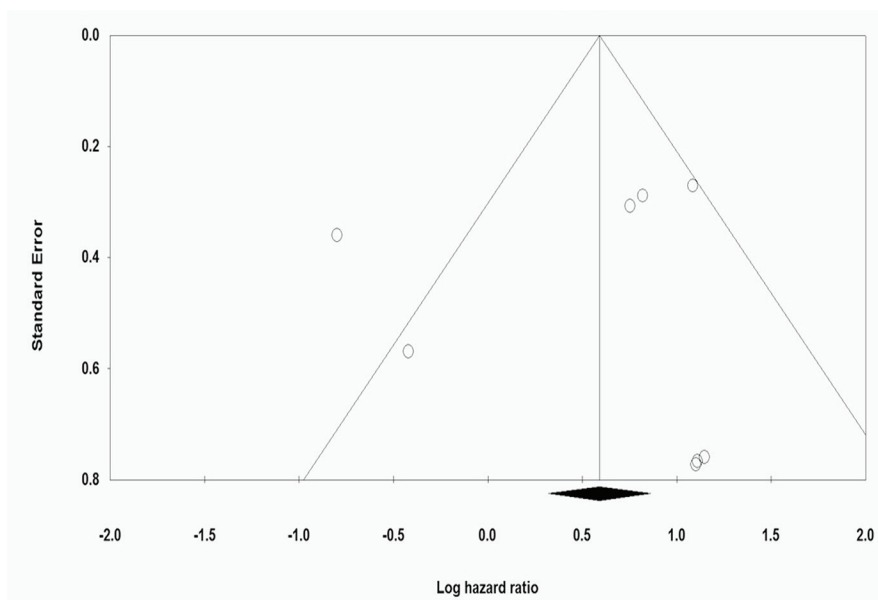

Figure 5. Funnel plot analysis to detect publication bias for the expression of SLUG. The horizontal axes represent the 95% confidence limits of logarithmic hazard ratio and vertical axes represent standard error of logarithmic hazard ratio.

Table 1. Baseline characteristics of the selected studies

EMT-TF	First Author	Year	Country	Case	Tumour site	Method	TFP (%)	Outcome	FT (M)	Ref	NOS
TWIST1	Zhao M	2013	China	200	Breast	IHC	75.5	OS	NT	6	6
	Soini Y	2011	Finland	388	Breast	IHC	52.4	OS	NT	7	6
	Jiang W	2012	China	137	Lung	IHC	38	OS	39	8	8
	Hui L	2013	China	120	Lung	qRT-PCR	38.3	OS	30.8	9	7
	Zhu DJ	2015	China	95	Colorectal	IHC	62.4	OS	NT	10	6
	Yusup A	2017	China	75	Colorectal	IHC	54.7	OS	NT	11	5
	Song YH	2014	Japan	53	Bladder	IHC	49.0	OS	25	12	8
	Lee KW	2012	Korea	165	Esophageal	IHC & qRT-PCR	51.5	DFS	115	13	7
	Gao XH	2013	China	37	Gastric	IHC	55.9		NT	14	6
	Zhao XL	2011	China	97	Liver	IHC	43.3	OS	NT	15	5
	Kim K	2014	Korea	123	Ovarian	IHC	28.5	OS	49	16	8
SNAIL1	Muenst S	2013	Switzerland	1043	Breast	IHC	25.4	OS	69.9	17	7
	Yang Z	2015	CHina	125	Breast	IHC	38.4	DFS & OS	89	18	8
	Zhou ZJ	2014	China	417	Liver	IHC	49.8	OS	NT	19	6
	Zhao N	2012	China	97	Liver	IHC	58.8	OS	NT	20	6
	Merikallio H	2012	Finland	279	Lung	IHC	21	OS	NT	21	6
	Shin NR	2012	Korea	314	Gastric	IHC	59.1	OS	51.4	22	7
	Kroepil F	2013	Germany	251	Colorectal	IHC	76	OS	NT	23	6
	Yu Q	2010	China	120	Bladder	IHC	62.5	OS	30	24	7
	Galvan JA	2014	Spain	134	Pulmonary NET	IHC	54.5	OS	90	25	8
SLUG	Liu T	2012	China	441	Breast	IHC	78	DFS & OS	60	26	8
	Atmaca A	2015	Germany	49	Lung	qRT-PCR	48.97	OS	NT	27	5
	Hasan R	2013	USA	152	Esophageal	IHC	70.3	OS	NT	28	7
	Uchikado Y	2012	Japan	164	Gastric	IHC	29.9	OS	35	29	8
	Zhang L	2013	China	119	Liver	IHC	59	OS	NT	30	5
	Toiyama Y	2013	Japan	181	Colorectal	IHC	50.82	OS	40	31	7
	Yu Q	2010	China	120	Bladder	IHC	62.5	OS	30	24	8
	Gu A	2016	China	60	Ovarian	IHC	73.3	OS	62.5	32	8
ZEB1	Montserrat N	2011	Spain	75	Breast	IHC	19	OS	46	33	7
	Goscinski MA	2015	China	176	Esophageal	IHC	64.9	OS	NT	34	5
	Okugawa Y	2011	Japan	134	Gastric	IHC	37.81	OS	23	35	7
	Hashiguchi M	2013	Japan	108	Liver	IHC	21.3	OS	48.4	36	5
	Zhou YM	2012	China	110	Liver	IHC	65.4	OS	NT	37	5
	Kurahara H	2012	Japan	76	Pancreas	IHC	25	OS	NT	38	6
	Guo C	2017	China	118	Colorectal	IHC	66.9	OS	18	39	7
	Li X	2016	China	238	Ovarian	IHC	32.8	OS	NT	40	6

EMT-TFs: EMT inducing transcription factors; IHC: Immunohistochemistry; qRT-PCR: quantitative real time- polymerase chain reaction; OS: overall survival; DFS: disease-free survival; TFP: EMT-TF positive; FT(M): follow-up time (months); NOS: Newcastle- Ottawa quality assessment scale; NT: not reported

standing question of whether these various inducers of EMT function independently or in combination or this function is facilitated by a common mediator. Recently, a study has shown the central role of AMP –activated protein kinase (AMPK) in mediating EMT through increased expression of TWIST1 and as a result, targeting AMPK, which might restrict cancer spread.[41]

Several experimental studies have associated the expression of EMT-TFs with the prognosis of different types of cancers, for instance breast, liver, lung, gastric, colorectal, bladder, esophageal, ovarian, pulmonary neuroendocrine tumor, and pancreatic cancers.[6-40] Thus, there has been a great interest in harnessing the potential of these EMT-TFs as cancer metastatic biomarker for the early diagnosis in order to improve patients' survival. Nevertheless, there is no widespread analysis to get a satisfactory conclusion on the correlation between EMT-TFs expression and cancer prognosis.

To our limited knowledge, this is the first meta-analysis exploring the clinical significance of the EMT-TFs (TWIST1, SNAIL1, SLUG, and ZEB1)

in various types of carcinoma. The present analysis involving 36 studies revealed that the over-expression of TWIST1, SNAIL1, SLUG, and ZEB1 in various cancers had profound independent effects on reducing the survival of patients. The pooled HR values implied that TWIST1 showed the most significant impact on the prognosis of carcinoma.

There are several investigations describing the association between EMT-TFs and cancer prognosis. SLUG expression was mediated by estradiol and ER-α, which lead to the poor prognosis in patients with breast cancer. A meta-analysis demonstrated that the SNAIL1 expression was higher in gastric cancer tissues than that in para-carcinoma tissues and normal tissues with the pooled Odds ratio (OR) values of respectively 6.15 (95 % CI = 4.70-8.05) and 17 (95 % CI = 10.08-28.67).[59] Kurahara et al. revealed that EMT contributes for the tumor progression and micro-metastasis in the regional lymph nodes by the up-regulation of ZEB1 and ZEB2; meanwhile, they reported that Mesenchymal to Epithelial transition (MET) is associated with epithelial phenotype in mature metastasis in the regional lymph nodes through the down-regulation of ZEB-1 and ZEB-2.[35] Another study reported that the co-expression of HIF-1α and TWIST1

reduced the E-cadherin levels through the p53 mediated regulation in ovarian endometrial cancer.[9]

The possible explanation for TWIST1 mediating higher prognosis in carcinomas, when compared to the other transcription factors, could be explained by the varied expression of EMT-TFs in different types of cancer with diverse tumor sites and different stages of cancer. Soini et al. reported that there was no expression of ZEB1, TWIST1, or SNAIL1 in epithelial tumor cell compartment of breast carcinoma. Alternatively, stromal cell compartment of the breast carcinoma revealed abundant expression of ZEB1 and TWIST1, yet not SNAIL1. Another study on bladder cancer depicted that the E-cadherin expression was down-regulated by TWIST1 and SLUG, yet not regulated by SNAIL1. Epigenetics might also be a causative factor for the differential expression of EMT markers in different tumor sites. A study showed that TWIST1 was highly hyper methylated in double negative (ER-negative and HER2/neu-negative) breast tumor than in human epidermal growth factor receptor (HER2/neu) or estrogen receptor (ER) positive tumors, exhibiting epigenetic differences among tumors.[42]

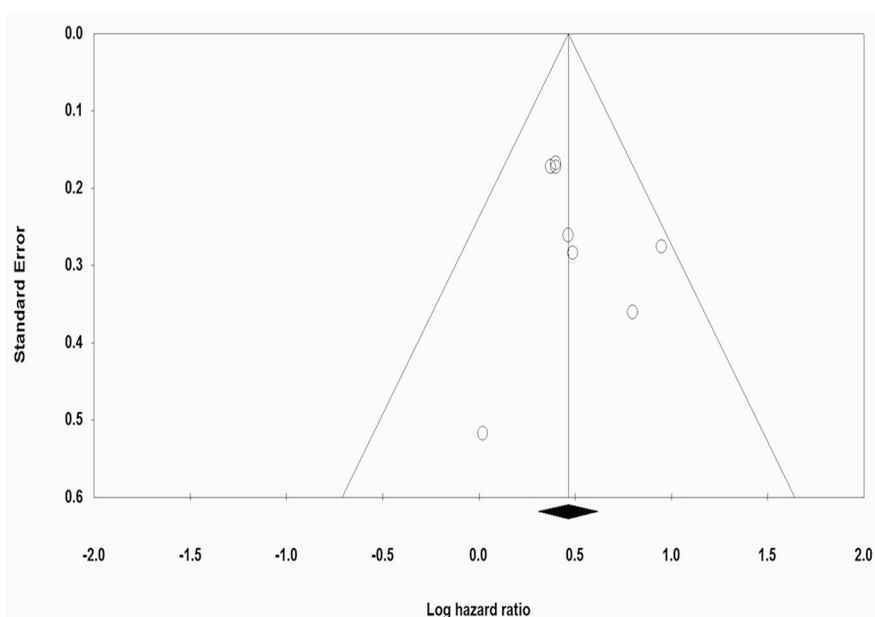Even though a lot of efforts have been made

Figure 6. Funnel plot analysis to detect publication bias for the expression of ZEB1. The horizontal axes represent the 95% confidence limits of logarithmic hazard ratio and vertical axes represent standard error of logarithmic hazard ratio.

to reduce heterogeneity in the study, there are a few limitations in this analysis. Primarily, the study included the expression data evaluated by both IHC and qRT-PCR. There have been reports suggesting the discrepancy between messenger ribo nucleic acid (mRNA) and protein expression, as IHC analysis appropriately detects the membranous expression of EMT-TFs (nucleus or cytoplasmic expression in tumor tissue), whereas mRNA studies detect the overall expression of all the cells in the tissues. Furthermore, there was non-uniform distribution in the IHC data due to the utilization of different kinds of primary antibody and antibody dilutions, IHC staining methods, and the independent cut-off values for each study that affected the overall sensitivity of the IHC technique. Accordingly, there is a need for standardized protocols in evaluating the expression levels of EMT-TFs in patient samples of all the studies. Secondly, there was no information regarding the patients' preoperative or postoperative treatments. Since the type of adjuvant and neoadjuvant therapy received by patients would also affect the prognosis of cancer, this is considered as a limitation.

Despite these limitations, the results of our findings demonstrated that the higher expression of EMT-TFs contribute for the cancer metastatic development and of all the other transcription factors, TWIST1 plays the leading role in the prediction of cancer.

Conclusion

In spite of remarkable advancements in clinical research, the mechanism by which tumor cells progress remains unclear. Among the various EMT-TFs, TWIST1 has been associated with poor prognosis of cancer; hence, a clear, detailed study should be undertaken to unravel the mysteries surrounding the molecular players of these TFs in signaling pathways. Our results indicated that TWIST1 might significantly affect the prognosis and survival of carcinoma patients. These findings are suggested to be employed as an effective biomarker for early diagnosis and metastasis therapy. Further experimental studies

are required to elucidate the functional role of these EMT-TFs in individual cancers and find new therapeutic interventions by targeting the EMT-TFs through RNAi or antisense technology.

Conflict of Interest

None declared.

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