Review Article

Significance of epithelial-to-mesenchymal transition inducing transcription factors in predicting distance metastasis and survival in patients with colorectal cancer: A systematic review and meta-analysis

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Background: The clinical relevance of epithelial-to-mesenchymal transition (EMT) in colorectal cancer (CRC) progression has been highlighted over the last decade. Several EMT-inducing transcription factors (EMT-TFs) have been implicated in the regulation of EMT, including Twist, Snail1, Slug, ZEB1, and ZEB2. Here, this meta-analysis aimed to predict the risk of distance metastasis and overall survival in CRC patients with high expression of EMT-TFs. **Materials and Methods:** All eligible studies were searched in PubMed, Scopus, and Web of Science databases. The search was carried out to include literatures published as late as September 1, 2018. In overall, 16 studies that investigated the relationship between EMT-TFs with distance metastasis and survival in CRC patients were included. In meta-analysis, a pooled hazard ratio (HR) and odds ratio (OR) were estimated for associations. **Results:** The results of this review indicated that expressions of all EMT-TFs are significantly correlated with poor overall survival in CRC. Moreover, there are a significant association between Twist (OR, 1.46; 95% confidence interval [CI], 1.03–2.09), Slug (OR, 3.43; 95% CI, 1.98–5.93), and ZEB2 (OR, 2.42; 95% CI, 1.09–5.40) expression with distance metastatic in CRC patients. **Conclusion:** These findings suggest that the overexpression of EMT-TFs plays a key role in increasing the risk of distance metastasis as well as decreasing overall survival in CRC patients.

Key words: Colorectal cancer, distance metastasis, epithelial–mesenchymal transition, overall survival, transcription factors

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INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent malignancies in the world.[1] Unfortunately, a significant number of patients with CRC progress to metastatic stage during the course of disease with a 5-year survival of <10%.[2] Over the past few years, there has been an increased interest in clinical and molecular prognostic factors in metastatic cancers. Knowing the prognostic factors is clinically important for designing appropriate treatment strategies based on an individual patient. It also gives insights into the biology of CRC metastasis, which can lead to emerging new therapeutic strategies. Accordingly, we aimed to conduct a meta-analysis study to find some prognostic factors related to distance metastasis and overall survival in CRC.

Metastasis is an enormously complex biological process involving different genes and biomolecules.[3,4] More recently, epithelial–mesenchymal transition (EMT) has been shown to be one of the key regulators of cancer metastasis. EMT is an essential process during cancer metastasis by which epithelial cells lose their

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adherent junctions and apical–basal cell polarity to form spindle-shaped cells that contribute to their ability to migrate as single cells.[5] The molecular changes during EMT are characterized and classified into four categories: (i) extracellular proteins, (ii) cell surface molecules, (iii) cytoplasmic, and (iv) nucleolus located biomolecules.[6]

Among these, nuclear EMT-inducing transcription factors (EMT-TFs) such as Slug, Snail1, Twist, ZEB1, and ZEB2 are often used as biomarkers of EMT.[7] Slug and Snail1 are zinc-finger TFs that directly suppress the expression of E-cadherin by binding to the specific E-boxes of E-cadherin's proximal promoter. E-cadherin plays a crucial role in cell adhesion and migratory capabilities.[8-10]

Twist is a protein that belongs to the family of basic helix-loop-helix proteins and functions as a TF.[11] Several studies demonstrated that the suppression of Twist in metastatic breast cells inhibits the metastatic process to the lung and the abnormal expression promotes the inhibition of E-cadherin expression causing the loss of cell–cell adhesion, activating the mesenchymal markers and cell motility.[12-14]

ZEB1 and ZEB2, members of the zinc-finger E-box-binding homeobox factor (ZEB) family, are transcriptional repressors that contain two widely separated clusters of C2H2-type zinc fingers that mediate their binding to paired CAGGTA/GE-box-like promoter elements.[15] These repressors induce EMT by suppressing the expression of E-cadherin and contribute to the progression of malignant cancer.[16,17]

Over the past few years, *in vitro* and *in vivo* observations have highlighted oncogenic functions of these EMT-TFs. However, the reports of EMT-TF expression in CRC and its association with prognosis are limited and controversial. The present meta-analysis, based on the published literatures, was aimed to investigate the relationship between EMT-TFs in CRC patients and major clinicopathological features, specifically the distance metastasis and overall survival.

MATERIALS AND METHODS

Search strategy and selection studies

A comprehensive systematic search was performed in the PubMed database, Web of Science, and Scopus to identify all the relevant studies before September 1, 2018; no lower date limit was used. The search was carried out on literature published in English. The main key terms for search were based on the research questions, such as: "Twist and metastatic colorectal neoplasm" or "Twist and colorectal cancer survival" or "Twist and stage IV colorectal cancer" or "Twist and distance metastasis." Furthermore, we repeated this search for other EMT-TFs including Slug, Snail1, ZEB1, and ZEB2. "AND" was used to connect the main research terms and the word of "OR" was used to incorporate synonym words.

Exclusion and inclusion criteria

Exclusion criteria in this meta-analysis were as follows: (i) reviews, case–controls, letters or experiments on cell lines, and animal models; (ii) studies evaluating the gene expression of EMT-TFs measured by real-time polymerase chain reaction (RT-PCR), microarray, or fluorescence *in situ* hybridization in CRC. Eligibility criteria were the assessment of Twist, Slug, Snail1, ZEB1, and ZEB2 expression by immunohistochemistry (IHC) and availability of metastatic and/or survival data. The associations of each EMT-TF with distance metastasis and with overall survival were declared in selected studies. In some studies, required data were not reported (NR). To provide proposed data, it was contacted through E-mail with the corresponding authors. In the case of no reply, the study was omitted from our meta-analysis study.

Distance metastasis was characterized as one of the features listed below: (1) "M0, M1" or (2) "vascular invasion" or (3) "neural invasion" or (4) "stage IV." The M refers to whether the cancer has metastasized (M0, no evidence of metastasis; M1, metastasis to distant organs). Stage IV is another staging system to describe the spread of cancer to distant parts of body.

Data collection and study assessment

All the selected articles were analyzed by two investigators (NA and AK), and any inconsistency or disagreement in the research process was resolved through consultations. If there were any conflicts, it would be referred to a third methodologist. In our study, sample size, technique of evaluation, and suitable statistical estimation of hazard ratio (HR) were considered as quality assessment criteria.

The electronic investigation was supplemented by a hand-search of relevant articles from the reference lists to ensure that all the studies could be identified. Finally, the following details were extracted: first author's name, year of publication, sample size, antibody used for the IHC, geographical location, metastatic stage, overall survival, and follow-up time. If the above information was not mentioned in the original study, the item was treated as "NR."

Statistical analysis

The heterogeneity tests examine the null hypothesis that all studies are evaluating the same effect. For assessing the heterogeneity of data, we used the I^2, tau square, and Cochran Q tests. A $P < 0.05$ and $I^2 < 50\%$ represented

good homogeneity. If there was a heterogeneity in the data, we used a random effect.[18] The association between overexpression of EMT-TFs and distance metastasis in CRC was analyzed using odds ratios (ORs). The association between the overexpression of EMT-TFs and the overall survival of CRC was evaluated using HR. A funnel plot was used to detect publication bias. It is a graphical representation of the size of the study plotted against the effect size. All analyses were conducted using Review Manager Version 5.3 (Cochrane Collaboration, Copenhagen, Denmark).

RESULTS

Literature search

Figure 1 shows the schematic diagram for search and selection of the included studies in this meta-analysis.

Study and patient characteristics

In this review, we analyzed the relationship between EMT-TF expressions with distance metastasis as prognostic indices. The five EMT-TFs were Twist, Slug, Snail1, ZEB1, and ZEB2. Overall, 16 studies were included in this analysis. For analysis of the relationship of Twist with distance metastasis, we entered 7 studies and it was 4, 7, and 1 for Slug, Snail1, and ZEB2, respectively. There was no previous study on the relationship of ZEB1 expression with distance metastasis. For analysis of the relationship of Twist with overall survival, 4 studies were entered. Moreover, 2, 3, 1, and 1 studies were entered for Slug, Snail1, ZEB1, and ZEB2, respectively.

The variables from 16 relevant studies are summarized in Table 1 according to the first author's name, year, and sample size, primary antibody used for IHC, population, distance metastasis, overall survival, and follow time for each study.

Analysis of epithelial–mesenchymal transition-inducing transcription factor expression with distance metastasis as prognostic indices of colorectal cancers

The pooled OR was estimated for relationship between Twist and distance metastasis according to a fixed random-effects model. We found a significant OR value of 1.46 (95% confidence interval [CI], 1.03–2.09) [Figure 2a].

Figure 2b shows the relationship between Slug and distance metastasis according to a random-effects model. We found a significant OR value of 3.43 (95% CI, 1.98–5.93).

The association between Sanil1 expression and distance metastasis was evaluated in 7 studies. For indicating this association, we used a random-effects model and the pooled OR which was presented in a forest plot [Figure 2c]. We found an OR value of 0.58 (95% CI, 0.32–1.05). The result of this meta-analysis showed no significant relationship between Snail1 and distance metastasis. Kroepil *et al*. evaluated the 251 patients with CRCs, and they were NR any metastatic patients in terms of snail expression categories.[29]

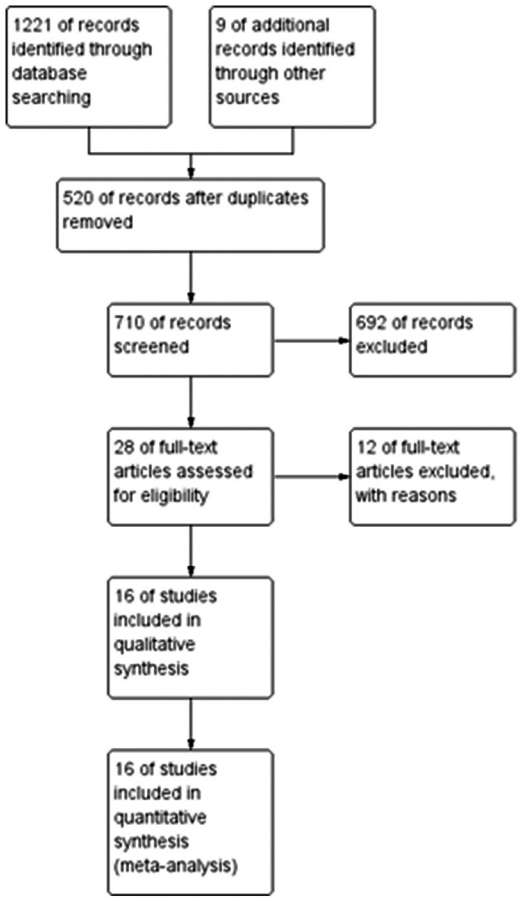

Figure 1: Flowchart of study search and selection process

The relationship between ZEB2 and distance metastasis was evaluated in one study. The results of this study showed that the relationship between ZEB2 and distance metastasis was significant (OR, 2.42; 95% CI, 1.09–5.40).[34]

Publication bias

We used a graphical presentation for publication bias in the case of relationship between Twist and distance metastasis. The shape of the funnel plot provided no statistical evidence for publication bias [Figure 3].

Analysis of epithelial–mesenchymal transition-inducing transcription factor expression with overall survival of colorectal cancers

Totally, 10 studies were entered for analysis of survival. Using a random-effects model, our meta-analysis showed that positive Twist expression is negatively correlated with overall survival in patients with CRC (HR, 2.92; 95% CI,

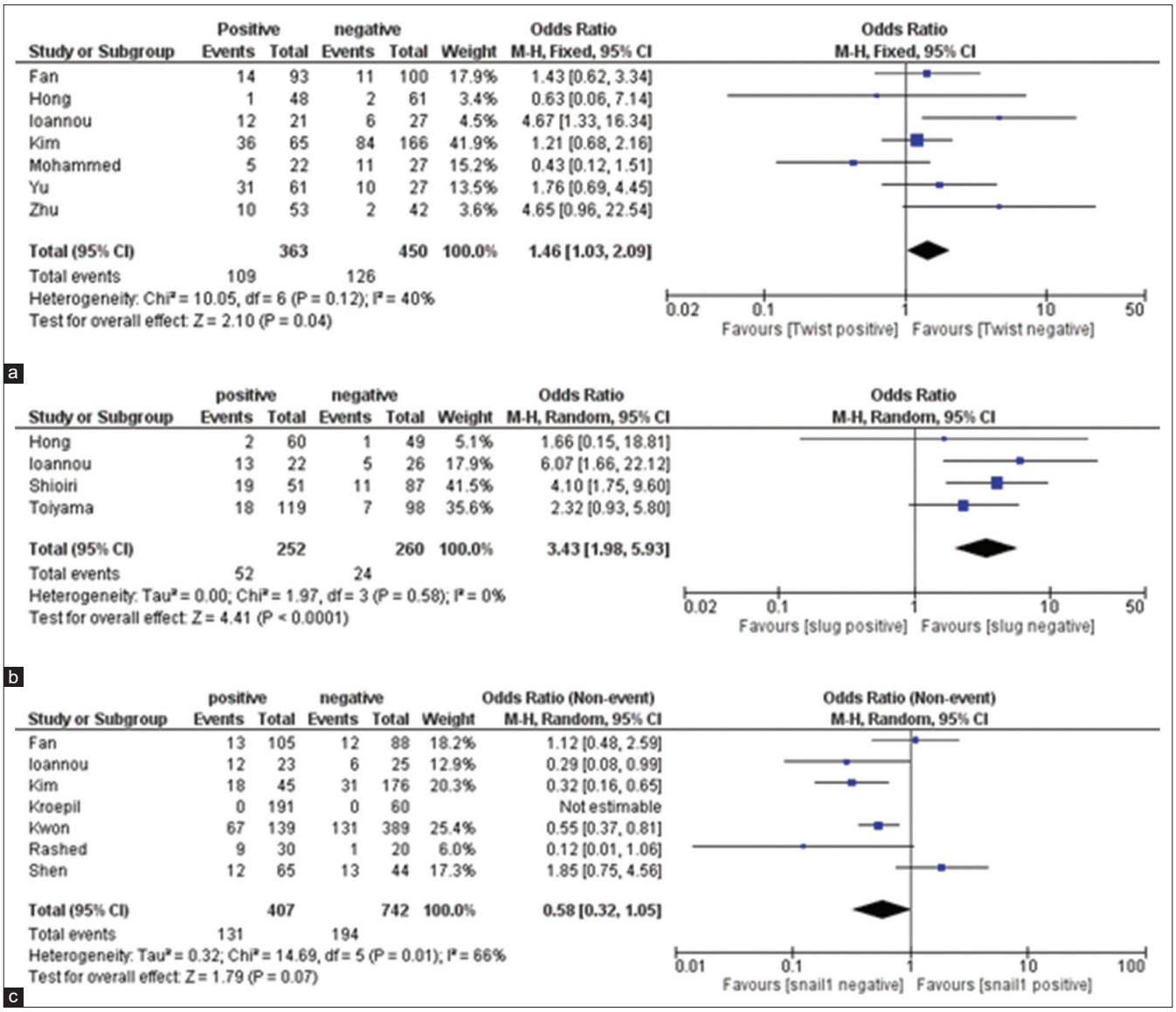

Figure 2: Meta-analysis results for expression of (a) Twist, (b) Slug, (c) Snail1, and distance metastasis risk in colorectal cancers

1.90–4.48) [Figure 4a]. Hong and Lim declared that Twist expression did not influence overall survival in colorectal adenocarcinoma.[20] We did not access these data; therefore, this study was not included in our meta-analysis study.

Similarly, Slug-positive expression was significantly associated with worse overall survival (HR, 2.30; 95% CI, 1.38–3.84) [Figure 4b].

Our meta-analysis showed that EMT-TFs including Snail1 had a significant association with overall survival (HR, 1.99; 95% CI, 1.51–2.62) [Figure 4c]. Kroepil *et al*. identified no prognostic impact of Snail1 expression on overall survival. This result was not included in our study because we did not access their row data.[29] For analysis of ZEB2 and ZEB1 with overall survival, we had found a single study for each. In the study of Kahlert *et al*., there was a significant relationship between ZEB2 and survival (HR, 2.48; 95% CI, 1.46–5.30),[34] and in the other study by Wu *et al*., the HR was 3.17 (95% CI, 1.64–6.13).[33]

DISCUSSION

The EMT is a complex biological process controlled by several transcriptional regulators including Twist, Slug, Snail1, ZEB1, and ZEB2. Many experimental studies showed that EMT-positive status is a significant predictor of distance metastasis in different types of tumors.[35] Moreover, the recent data have linked the expression of EMT-TFs with overall survival.[36,37] In CRC, 85% of resected specimens have moderate-to-strong Twist expression, which is notably more than either Snail1 or Slug.[27,29] Slug and ZEB1 expression is significantly correlated with lower expression of E-cadherin which is responsible for cell adhesion,[27,38]

Table 1: The main characteristics of all studies

EMT-TF	Author (reference)	Years	Sample size	Primary antibody	Population	Distant metastasis	Overall survival	Follow time (months)
Twist	Fan *et al*.[19]	2013	193	ab50887, Abcam, CA, USA	Chinese	IV	-	NR
	Hong and Lim[20]	2009	109	Santa Cruz, CA, USA	Korean	M0, M1	+	NR
	Ioannou *et al*.[21]	2018	48	Santa Cruz, CA, USA	Greece	Vascular invasion	-	NR
	Kim *et al*.[22]	2014	231	Novus Biologicals	Korean	Vascular invasion	+	71
	Mohammed *et al*.[23]	2015	49	ab50581, Abcam, UK	Egypt	M0, M1	-	NR
	Yu *et al*.[24]	2013	93	Abnova	Chinese	M0, M1	+	32
	Zhu *et al*.[25]	2015	95	Santa Cruz, CA, USA	Chinese	M0, M1	+	2005-2014 years (~108)
Slug	Hong *et al*.[26]	2008	109	Santa Cruz, CA, USA	Korean	M0, M1	-	NR
	Ioannou *et al*.[21]	2018	48	Santa Cruz, CA, USA	Greece	Vascular invasion	-	NR
	Shioiri *et al*.[27]	2006	138	Santa Cruz, CA, USA	Japanese	M0, M1	+	5.11 years (5.11×12)
	Toiyama *et al*.[28]	2013	208	Cell Signaling Technology, MA, USA	Japanese	Distant metastasis	+	40
Snail1	Fan *et al*.[19]	2013	193	ab70983; Abcam, CA	Chinese	IV	-	NR
	Ioannou *et al*.[21]	2018	48	Santa Cruz, CA, USA	Greece	Vascular invasion	-	NR
	Kim *et al*.[22]	2014	231	Novus Biologicals	Korean	Vascular invasion	+	71
	Kroepil *et al*.[29]	2013	251	Ab17732, Abcam	Germany	M0, M1	-	NR
	Kwon *et al*.[30]	2015	528	Abcam, UK	Korean	Perineural invasion	+	NR
	Rashed *et al*.[31]	2017	50	Santa Cruz, CA, USA	Egypt	M0, M1	-	NR
	Shen *et al*.[32]	2017	109	NR	Chinese	M0, M1	+	NR
ZEB1	Wu *et al*.[33]	2016	145	NR	Taiwan	-	+	~48
ZEB2	Kahlert *et al*.[34]	2011	175	Novus Biologicals, USA	Germany	M0, M1	+	124

EMT-TF=Epithelial-to-mesenchymal transition-inducing transcription factor; NR=Not reported

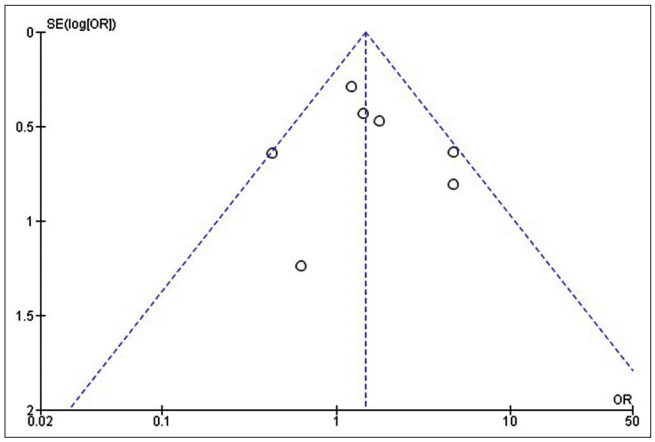

Figure 3: Funnel plot of Twist-related studies to detect publication bias. Each point represents a single study for the specified association; the vertical axis represents the standard error of the logarithmic odds ratio, and the horizontal axis represents the odds ratio limits

and upregulation of ZEB1 and ZEB2 at the invasion front both correlate with shorter survival times.[34] Furthermore, a significant correlation has been reported between Slug and vimentin expression, and upregulation of Slug has emerged as an independent prognostic factor and a predictive marker of lymph node metastasis and sprouting angiogenesis in CRC patients.[28]

To evaluate the potential of EMT markers that could be used for risk stratification for patients with CRC, a systematic review of studies (n = 30) was conducted. In that study, at least one of a selection of EMT markers in primary tumors and patient outcomes were measured. Fifteen of 30 studies (50%) reported at least one statistically significant result supporting a role for one of the selected EMT markers in identifying patients at risk for worse outcomes.[39]

Therefore, although the association of EMT-TF expression with the CRC prognosis has been explored for several years, the available data have not been comprehensively analyzed until now. To assess the possibility of translating EMT-TFs to colorectal prognosis, we studied published data concerning the expression of Twist, Slug, Snail1, ZEB1, and ZEB2 in CRC and their association with distance metastasis and overall survival. The definition of EMT-TF-positive expression was based on IHC analysis in all the eligible articles, as expressed as the percentage of positive cells or/and staining intensity.

In this study, for the first time, the HR results showed that the expression of Twist, Slug, Snail1, ZEB1, and ZEB2 was associated with worsening survival in CRC, in which Twist might serve as the most significant prognostic marker for CRC. Therefore, this study provided compelling evidence that the overexpression of Twist, Slug, Snail1, ZEB1, and ZEB2 may contribute to the progression of CRC, and further study could provide useful guidelines for physicians to improve follow-up plans for CRC patients. In

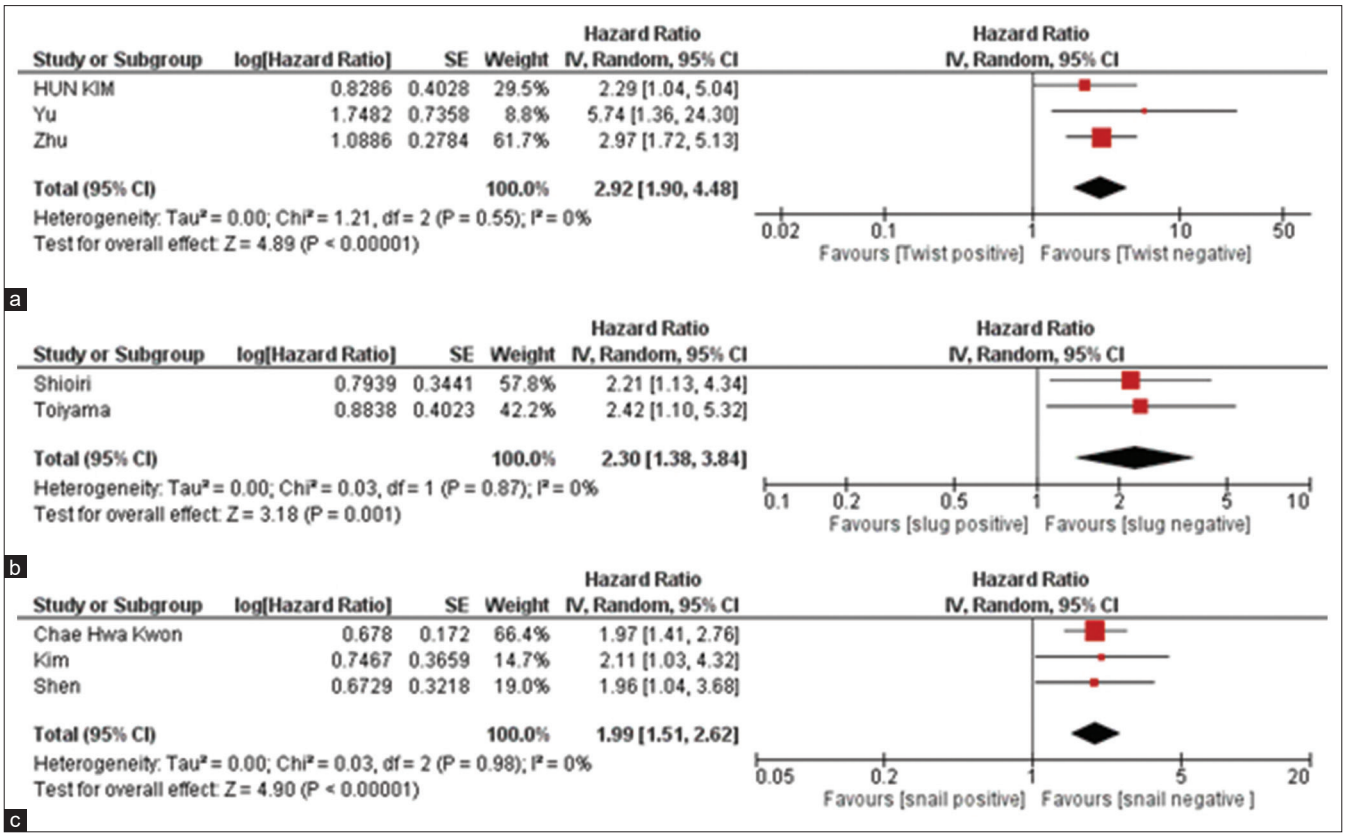

Figure 4: Forest plot for the association of expression of (a) Twist, (b) Slug, and (c) Snail1, with overall survival in colorectal cancer patients using pooled hazard ratio

a similar study, Imani *et al*. showed that the expression of Twist1, Snail1, and especially Slug has an association with advanced stage of metastatic breast cancer and worsening survival. The increased risk of metastatic breast cancer was less associated with ZEB1 expression.[6] In another meta-analysis study by Wan *et al*., the association of EMT-TF overexpression with a potential poor prognostic factor in patients with hepatocellular carcinoma was reported.[40]

Here, we point out the shortcomings of our findings and outline our comments for further research. First, the studies published in languages other than English are not included in this study. The second inevitable limitation lies in the evaluation technique, which was IHC in our study. To make the data more homogeneous and strengthen the statistical analysis, we excluded the experiments that used techniques such as RT-PCR, microarray, fluorescence *in situ* hybridization, and whole-exome sequencing, though IHC technique has its own limitations. Different IHC protocols with antibodies from different sources might affect the results. Moreover, there is not a standard cutoff value for positive expression of EMT-TFs which is dependent on the staining score and may be different in various experiments.

One of the other reasons for heterogeneity other than technique might be the patient's differences. We did not have information about the onset, type, and duration of adjunctive therapies. Certainly, the results of this meta-analysis should be interpreted cautiously because there might be underlying heterogeneity.

Despite these limitations, the data of the present meta-analysis suggest that Twist, Snail1, ZEB1, and ZEB2 overexpression might be critical markers in prognosis of CRC. In addition, we especially evaluated the risk of high Twist, Slug, Snail1, ZEB1, and ZEB2 levels and the worsening survival in CRC patients.

CONCLUSION

Taken together, analyzing the importance of the EMT-TFs in the acquisition of metastatic properties and its link with overall survival exposes novel therapeutic opportunities in CRC patients. Preventing or inducing a reversible switch in EMT-TF expression in CRCs may be a promising approach to target CRC.

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Conflicts of interest
There are no conflicts of interest.

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