

# Value of Biomarkers in Liver Cancer EMT model under different interventions: A meta-analysis

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## Research Article

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# Abstract

## Background

There are various interventions to establish the Liver cancer epithelial-mesenchymal transition (EMT) models. However, the ideal biomarkers for unique model are not well established. Further studies are necessary to evaluation of effective EMT biomarkers under different interventions in vitro studies. A meta-analysis was performed to evaluate the performance of different biomarkers in HepG2 cells during EMT under multiple interventions.

## Methods

PubMed, Web of Science, Embase, the China National Knowledge Infrastructure (CNKI), the China Biology Medicine disc (CBM), Wan Fang Data, and VIP databases were systematically searched from inception to June 14, 2020 by two independent reviewers.

## Results

A total of 58 studies were included in the meta-analysis. Our study showed that E-cadherin responds well to the intervention of medication, genetic intervention, gene knockout/knockdown, hypoxia, and other tumor microenvironments, as well as non-coding RNA (ncRNA) overexpression and silencing. N-cadherin can effectively evaluate the intervention effect of medication, genetic intervention, hypoxia and other tumor microenvironments, as well as ncRNA overexpression. Vimentin reflects the effects of medication, pro-EMT genetic intervention and gene knockout/knockdown, anti-EMT ncRNA overexpression and anti-EMT ncRNA silencing and hypoxia. Snail only responds to the intervention of anti-EMT genetic intervention and gene knockout/knockdown, tumor microenvironments other than hypoxia, anti-EMT ncRNA overexpression and ncRNA silencing.

## Conclusions

Our results shows that some medicine, some gene, microenvironment and some ncRNA can effectively induce/inhibit EMT process. E-cadherin, N-cadherin, Vimentin and Snail are effective biomarkers during this process. They respond differently to different intervention. Therefore, different biomarkers should be chosen under different intervention based on their performance.

## Background

Liver cancer is one of the deadliest malignancies. According to the global cancer statistics released by the World Health Organization in 2018, about 800,000 people die from liver cancer every year and the global incidence rate is increasing rapidly [1]. China has the most cases of liver cancer resulting in about

100,000 deaths every year [2]. Moreover, the majority of cases are diagnosed at the locally or distantly advanced stage, which contributes to a high rate of postoperative relapse and metastasis [3].

Multiple studies [4–5] have shown that epithelial-mesenchymal transition (EMT) can promote the invasion and metastasis process by enhancing the separation of the original tumor mass and subsequent metastasis, thereby entering the bloodstream or lymphatic flow circulation. The most critical characteristics of cancer cells that undergo EMT is the downregulation of epithelial markers and upregulation of mesenchymal markers [6]. Several studies have targeted these biomarkers to promote or inhibit the EMT process. For example, miR-125a-5p downregulates the epithelial marker E-cadherin and upregulates the stromal markers N-cadherin and Vimentin to promote EMT [7]. CD47 regulates E-cadherin, N-cadherin, and Snail to induce EMT [8].

There are several biomarkers of EMT, among which E-cadherin is the most critical epithelial marker and the main component of cell adhesion [9]. N-cadherin induces the transformation of epithelial cells into fibroblasts during cancer development, making cells more motile and invasive [10], and Vimentin (an intermediate filamentous protein) plays an important role in the formation of mesenchymal cells [11]. Different studies have used different combinations of biomarkers to evaluate the effect of EMT after intervention. Meng J et al. [12] used E-cadherin, N-cadherin, and Vimentin to evaluate ubiquitin-specific protease 5 (USP5) intervention on EMT. Wang Y et al. [13] used E-cadherin and Vimentin to determine the effect of lncRNA-CASC2 intervention on EMT. However, the underlying reason for choosing these markers was not explained. Further investigation [14] suggested that different types of EMT exhibit different biomarker profiles. The process of EMT may be regulated by several different signaling pathways such as Wnt/ $\beta$ -catenin, Notch, Hedgehog, and receptor tyrosine kinase-mediated signaling pathways [15]. Therefore, a comprehensive evaluation of effective EMT biomarkers under different interventions is imprecise for guiding future in vitro studies.

Meta-analysis is an effective way to comprehensively summarize the available evidence [16]. A large number of in vitro EMT studies have been performed in the recent years [17–18]. Among them, HepG2 cells are the most commonly used cell line for in vitro studies because of their constant and stable phenotype, easy acquisition, and easy treatment [19]. Several studies have subject this cell line to different kinds of treatments with drugs, genes, ncRNAs, and microenvironmental changes [20–22]. In this study, we evaluated the effect of these interventions on the four most common EMT biomarkers (E-cadherin, N-cadherin, Vimentin, and Snail) to evaluate their effectiveness and selection for future studies.

## Methods

This meta-analysis followed the PRISMA guidelines strictly [23] and the details of the interventions are provided in Supplemental 1.

## Study selection

PubMed, Web of Science, Embase, CNKI, CBM, Wan Fang Data, and VIP databases were systematically searched since inception till June 14, 2020. The retrieval method of combining subject words and free words was carried out. The following terms were used for the search: “Hepatocellular Carcinoma” or “Liver Cancer” or “Liver Neoplasms”, “Epithelial-Mesenchymal Transition” or “EMT,” and “Hep G2 Cell” or “Hepatoblastoma G2 Cell Line.” The search strategies for all the databases are described in Supplemental 2. The original literature were used for study.

## **Inclusion and exclusion criteria**

Studies that fulfilled the following criteria were included: (1) in vitro studies that were published in both English and Chinese; (2) the HepG2 cell line was used, under any intervention; and (3) expression level of the biomarker were measured by western blotting (WB). Studies were excluded from meta-analysis if (1) the quality of experiments was poor, with no specific experimental methods and steps, lack of information pertaining to cell sources, and culture details; (2) the experiments were repeated less than three times and no mean and standard deviation values could be obtained after contacting the corresponding author; and (3) the full text of the published articles was not available.

## **Data extraction**

An MS Excel data extraction table was created by two independent reviewers (Jing Yan and Bei Xie). The following information was obtained from the studies: (1) authors, published year; (2) the name, dose, and duration of the intervention; (3) source of the cell line and culture medium; (4) the expression of EMT biomarkers including E-cadherin, N-cadherin, Vimentin, and Snail.

## **Quality assessment**

Two independent reviewers (Yan Jing and Shuli Zou) performed quality evaluation of the collected data. To assess the risk of bias in vitro cell-based studies, we comply with the standard established by Golbach LA et al. [24]. Based on our study, the following 11 factors were evaluated: cell source, intervention time, intervention dose, cell culture, cell viability assessment, experimentation process, measurement process of results, randomization and blinding of study, control, standardized reagent and instrument. Based on the above information, the study was rated as “high quality” “low quality” or “unclear quality.”

## **Data analysis**

Stata15 was used for performing meta-analysis. The mean difference (MD) was calculated for continuous variables. The standardized mean difference (SMD) was calculated to analyze continuous data. 95% confidence interval (CI) was calculated for every variables. Heterogeneity among studies was tested by  $I^2$ . An  $I^2$  value of  $\leq 50\%$  indicated no significant heterogeneity and the data were combined by a fixed-effect model; otherwise, a random-effect model was used. P-value of  $<0.05$  was considered as statistically significant.

To explore potential sources of heterogeneity and obtain further information, we performed a subgroup analysis based on different interventions and effect.

(1) Drug: Traditional Chinese medicine (TCM), Western medicine group A (pro-EMT drug) and group B (anti-EMT drug).

(2) Gene: Genetic intervention group A (pro-EMT genetic intervention) and group B (anti-EMT genetic intervention), gene knockout or knockdown.

(3) Tumor microenvironment: hypoxia, others;

(4) ncRNA: Overexpression of ncRNAs group A (pro-EMT genetic intervention) and group B (anti-EMT genetic intervention), ncRNA silence group A (pro-EMT genetic intervention) and B (anti-EMT genetic intervention).

## Results

### Literature search

As shown in Figure 1, a total of 849 studies were identified with research method mentioned above. After removing duplicates, 660 studies were considered for title and abstract screening. Of these studies, 266 were selected for full-text screening and 58 were finally included in the meta-analysis.

### Study Characteristics

A total of 58 studies were identified (including 63 individual experiments). The distribution of studies involving different interventions was as follows: 17 experiments used drug (9 TCM, 8 Western medicine), and 22 experiments used genetic interventions (14 gene overexpression, 8 gene knockout/down), 9 experiments used tumor microenvironment interventions (5 used hypoxia, 4 used other methods), 15 experiments used ncRNA interventions (9 ncRNA overexpression, 6 ncRNA silence). Biomarker utilization was as follows: E-cadherin was reported in 63 experiments, Vimentin was reported in 47 experiments, N-cadherin was reported in 37 experiments, and Snail was reported in 21 experiments. The basic characteristics of the studies are given in Supplemental 3.

## Risk of Bias Characteristics

The results of the risk of bias assessment are presented in Supplemental 4. Cell source was not reported in 9.52% (6/63) studies, 14.29% (9/63) studies did not implement the cell vitality assay, 38.1% (24/63) studies did not report intervention time, 65.08% (41/63) studies did not report intervention concentration, and 1.59% (1/63) studies did not report the cell culture process.

## Meta-analysis Results

### Drug

Seventeen experiments from 14 studies [25–38] were identified with drug intervention. All 17 experiments reported changes in the expression of E-cadherin (Figure 2A). Nine experiments reported changes in the

expression of N-cadherin (Figure 3A), 15 experiments reported changes in the expression of Vimentin (Figure 3B), 5 experiments reported changes in the expression of Snail (Figure 3C).

(1) E-cadherin: The expression of E-cadherin in the TCM intervention group (SMD = 4.10, 95% CI [2.51, 5.68],  $P < 0.05$ ) and Western medicine anti-EMT group (SMD = 4.30, 95% CI [2.44, 6.16],  $P < 0.05$ ) increased significantly compared with control, whereas the expression of E-cadherin in the Western medicine pro-EMT group (SMD = -4.62, 95% CI [-8.04, -1.20],  $P < 0.05$ ) significantly decreased compared with control.

(2) N-cadherin: The expression of N-cadherin in the TCM intervention group (SMD = -5.56, 95% CI [-9.24, -1.88],  $P < 0.05$ ) and the Western medicine anti-EMT group (SMD = -9.11, 95% CI [-18.05, -0.17],  $P < 0.05$ ) were significant lower than control group. The expression of N-cadherin of Western medicine pro-EMT group (SMD = 8.56, 95% CI [2.56, 14.56],  $P < 0.05$ ) was significantly higher than control group.

(3) Vimentin: The expression of Vimentin in the TCM intervention group (SMD = -6.77, 95% CI [-9.18, -4.35],  $P < 0.05$ ) and the Western medicine intervention group (SMD = -4.26, 95% CI [-6.15, -2.37],  $P < 0.05$ ) was significantly lower than the control group.

(4) Snail: The expression of Snail in the TCM intervention group (SMD = -10.04, 95% CI [-21.18, 1.11],  $P > 0.05$ ) and Western medicine intervention group (SMD = -5.05, 95% CI [-10.18, 0.08],  $P > 0.05$ ) were lower than that in the control group, but without significant difference.

## Gene

Twenty two experiments used Genetic intervention [39–60]. As shown in Figure 2B, all these studies reported changes in the expression of E-cadherin. Fourteen experiments reported changes in the expression of N-cadherin (Figure 3D), 16 experiments reported changes in the expression of Vimentin (Figure 3E), and 10 experiments reported changes in the expression of Snail. Forest diagrams are presented in Figure 3 (Figure 3F).

(1) E-cadherin: The expression of E-cadherin in the pro-EMT genetic intervention group (SMD = -4.76, 95% CI [-6.75, -2.76],  $P < 0.05$ ) was significantly lower than the control group. The expression of E-cadherin in the anti-EMT genetic intervention group (SMD = 6.16, 95% CI [2.54, 9.78],  $P < 0.05$ ) and the gene knockout/knockdown intervention group (SMD = 4.58, 95% CI [2.79, 6.37],  $P < 0.05$ ) was significantly higher than the control group.

(2) N-cadherin: The expression of N-cadherin in the pro-EMT genetic intervention group (SMD = 6.16, 95% CI [2.80, 9.52],  $P < 0.05$ ) was significantly higher than the control group. The expression of N-cadherin in the anti-EMT genetic intervention group (SMD = -4.41, 95% CI [-7.67, -1.16],  $P < 0.05$ ) was significantly lower than the control group.

The expression of N-cadherin in the gene knockout/knockdown group (SMD = -2.84, 95% CI [-6.04, 0.36],  $P > 0.05$ ) was lower than that in the control group, but not statistically significant.

(3) Vimentin: The expression of Vimentin in the pro-EMT genetic intervention group (SMD = 5.04, 95% CI [3.13, 6.95],  $P < 0.05$ ) was significantly higher than the control group. Vimentin expression in the gene knockout/knockdown intervention group (SMD = -4.84, 95% CI [-7.33, -2.36],  $P < 0.05$ ) was significantly lower than that in the control group.

The expression of Vimentin in the anti-EMT genetic intervention (SMD = -2.71, 95% CI [-5.78, 0.36],  $P < 0.05$ ) was lower than that in the control group. However, there was no significant difference.

(4) Snail: The expression of Snail in the anti-EMT genetic intervention group (SMD = -5.48, 95% CI [-9.59, -1.38],  $P < 0.05$ ) and the gene knockout/knockdown intervention group (SMD = -8.49, 95% CI [-12.32, -4.65],  $P < 0.05$ ) was significantly lower than that the control group.

The expression of Snail in the pro-EMT genetic intervention (SMD = 0.88, 95% CI [-1.16, 2.92],  $P > 0.05$ ) was higher than that in the control group, but without significant difference.

## Tumor Microenvironment

Tumor microenvironmental intervention was reported in 9 experiments from 8 studies [26, 37, 46, 61–65]. Of these, as shown in Figure 2C, 9 experiments reported changes in the expression of E-cadherin, 4 experiments reported changes in the expression of N-cadherin (Figure 4A), 6 experiments reported changes in the expression of Vimentin (Figure 4B), and 3 experiments reported changes in the expression of Snail (Figure 4C).

(1) E-cadherin: The expression of E-cadherin in the hypoxia group (SMD = -5.26, 95% CI [-8.48, -2.05],  $P < 0.05$ ) and the other tumor microenvironment group (SMD = -4.27, 95% CI [-6.77, -1.77],  $P < 0.05$ ) was significantly lower than the control group.

(2) N-cadherin: The expression of N-cadherin in the hypoxia group (SMD = 10.98, 95% CI [0.26, 21.71],  $P < 0.05$ ) and the other tumor microenvironment group (SMD = 2.59, 95% CI [0.03, 5.16],  $P < 0.05$ ) was significantly higher than the control group.

(3) Vimentin: The expression of Vimentin in the hypoxia group (SMD = 5.8, 95% CI [0.57, 11.03],  $P < 0.05$ ) was significantly higher than the control group.

The expression of Vimentin in the other tumor microenvironment group (SMD = 5.21, 95% CI [-0.07, 10.49],  $P < 0.05$ ) was higher than that in the control group, but without significant difference.

(4) Snail: The expression of Snail in the other tumor microenvironment group (SMD = 24.89, 95% CI [0.80, 48.98],  $P < 0.05$ ) was significantly lower than the control group.

The expression of Snail in the hypoxia group (SMD = 6.64, 95% CI [-6.59, 19.86],  $P > 0.05$ ) was higher than that in the control group, but without significant difference.

## ncRNA

ncRNA intervention was found in 15 experiments from 15 studies [66–80]. Of these, as shown in Figure 2D, 15 experiments reported changes in the expression of E-cadherin, 12 experiments reported changes in the expression of N-cadherin (Figure 4D), 11 experiments reported changes in the expression of Vimentin (Figure 4E), and 6 experiments reported changes in the expression of Snail (Figure 4F).

(1) E-cadherin: The expression of E-cadherin in the ncRNA overexpression pro-EMT group (SMD = -7.08, 95% CI [-13.02, -1.13],  $P < 0.05$ ) and the ncRNA silence pro-EMT group (SMD = -2.27, 95% CI [-4.29, -0.24],  $P < 0.05$ ) and was significantly lower than the control group. The expression of E-cadherin in the ncRNA overexpression anti-EMT group (SMD = 3.28, 95% CI [1.45, 5.11],  $P < 0.05$ ) and the ncRNA silence anti-EMT group (SMD = 7.06, 95% CI [3.70, 10.42],  $P < 0.05$ ) was significantly higher than the control group.

(2) N-cadherin: The expression of N-cadherin in the ncRNA overexpression pro-EMT group (SMD = 7.68, 95% CI [0.09, 15.26],  $P < 0.05$ ) was significantly higher than that in the control group, whereas overexpression in the ncRNA anti-EMT group (SMD = -3.58, 95% CI [-6.70, -0.45],  $P < 0.05$ ) was significantly lower than that in the control group.

The expression of N-cadherin in the pro-EMT ncRNA silence (SMD = 8.14, 95% CI [-9.77, 26.05],  $P > 0.05$ ) was higher than that in the control group, whereas the expression of N-cadherin in the anti-EMT ncRNA silence (SMD = -6.05, 95% CI [-12.25, 0.14],  $P > 0.05$ ) was lower than that in the control group, but without significant difference.

(3) Vimentin: The expression of Vimentin in the ncRNA overexpression anti-EMT group (SMD = -6.59, 95% CI [-9.94, -3.23],  $P < 0.05$ ) and the ncRNA silence anti-EMT group (SMD = -9.80, 95% CI [-14.74, -4.86],  $P < 0.05$ ) was significantly lower than the control group.

The expression of Vimentin in the pro-EMT ncRNA overexpression (SMD = 9.80, 95% CI [-0.57, 20.17],  $P > 0.05$ ) and the pro-EMT ncRNA silence (SMD = 5.91, 95% CI [-0.02, 11.85],  $P > 0.05$ ) was higher than that in the control group, but without significant difference.

(4) Snail: The expression of Snail in the ncRNA silence pro-MET group (SMD = 11.03, 95% CI [0.26, 21.80],  $P < 0.05$ ) was significantly higher than the control group, whereas its overexpression in the ncRNA anti-EMT group (SMD = -7.32, 95% CI [-14.56, -0.07],  $P < 0.05$ ) and the ncRNA silence anti-EMT group (SMD = -6.40, 95% CI [-11.52, -1.28],  $P < 0.05$ ) was significantly lower than that the control group.

The expression of Snail in the pro-EMT ncRNA overexpression (SMD = 10.27, 95% CI [-4.69, 25.23],  $P > 0.05$ ) was higher than that in the control group, but without significant difference.

## Publication bias

Publication bias for the meta-analysis with regard to the expression of E-cadherin in the genetic intervention group, indicated that there was no obvious publication bias in the included literature (Egger test  $P = 0.96$ ).

## Discussion

To the best of our knowledge, this is the first meta-analysis study that explored the expression of biomarkers under diverse pro-EMT and anti-EMT interventions in HepG2 cells. It based on the pre-intervention and post-intervention self-control of HepG2 cells. Our study incorporated 58 studies with all different ways of interventions and contained more detailed information about experimental and detection process, and excluded the poor-quality articles that did not provide details about cell origin or experimental process. Primarily, we classified these 58 studies according to the interventions, and then subgrouped some intervention groups according to the inconsistent outcomes (enhancement or inhibition of EMT), thus systematically evaluated the effectiveness of biomarkers under interventions with different outcomes.

Our study showed that E-cadherin responds well to the intervention of medication, genetic intervention, gene knockout/knockdown, hypoxia, and other tumor microenvironments, as well as ncRNA overexpression and silencing. N-cadherin can effectively evaluate the intervention effect of medication, genetic intervention, hypoxia and other tumor microenvironments, as well as ncRNA overexpression. Vimentin reflects the effects of medication, pro-EMT genetic intervention and gene knockout/knockdown, anti-EMT ncRNA overexpression and anti-EMT ncRNA silencing and hypoxia. Snail only responds to the intervention of anti-EMT genetic intervention and gene knockout/knockdown, tumor microenvironments other than hypoxia, anti-EMT ncRNA overexpression and ncRNA silencing.

E-cadherin can better evaluate the effect of medicine, genes, microenvironment, and ncRNAs on EMT intervention. The reason may be that medicine, genes, microenvironment, and ncRNAs can directly or indirectly act on the signaling pathways to activate the upstream protein E-cadherin and therefore its expression. E-cadherin, the first member of the cadherin family, is an active inhibitor responsible for invasion and growth in many epithelial cancers [10]. Some studies have shown that E-cadherin, as a core protein, can change the adhesion activity on the cell by regulating signaling pathways including Wnt, PI3K, MAPK and Hippo in the action of tumor EMT to surface [81]. Decreased expression of E-cadherin weakens cell-cell adhesion which promotes the separation of tumor cells from the primary tumor mass, leading to invasion, proliferation, and metastasis of cancer cells [82]. It is well known that targeted drugs can directly act on tumor-associated signaling pathways. For example, Han et al. [34] reported in the meta-analysis that doxycycline significantly increased the activity of the PI3K/Akt signaling pathway and significantly inhibited the expression of E-cadherin. Another studies [83] found that after the activation of the PI3K/Akt signaling pathway, the expression of Snail, Slug, Twist, and other nuclear transcription factors was increased and that of E-cadherin was directly inhibited, thus promoting EMT. Similarly, regulation of other related pathways can also significantly decreases E-cadherin expression then induce EMT. Onder TT et al. [84] found that the expression of inducible transcription factors Zeb, Snail, and Twist could lead to the downregulation of E-cadherin. Gregory PA et al. [85] found that the miR-200 family and miR-205 could inhibit transcription factors ZEB1 and SIP1 and then cause the decreased expression of E-cadherin, thus inducing EMT. In addition, hypoxia can promote the EMT of tumor cells via HIF-1 $\alpha$  [86]. HIF-1 $\alpha$  is the upstream regulator of Snail [87]. It can directly induce the increase of snail expression. In

conclusion, E-cadherin can effectively reflect the EMT process in HepG2 cells under various interventions. Therefore, we recommend it to be the first choice in future in vitro studies as a EMT biomarker for medicine, gene, microenvironment, and ncRNA interventions in HepG2 cells.

Our study also showed that N-cadherin expression changes significantly to EMT process induced by medicine, genetic intervention, tumor microenvironment and ncRNA overexpression intervention. N-cadherin is a calcium-dependent single-chain transmembrane glycoprotein that mediates homotype and heterotype adhesion between cells [88]. Overexpression of N-cadherin changes cell polarity and inter-cell adhesion, making cancer cells more prone to metastasis [89]. A previous study showed that during EMT, cadherin changes from E-cadherin to N-cadherin, so the upregulated expression of N-cadherin is considered an important EMT biomarker [90]. N-cadherin expression in tumor cells was regulated by TGF- $\beta$ 1, Wnt/ $\beta$ -catenin, EGFR and NF-kappa B, etc [88]. Jiao, M et al. [63] found that the PI3K/Akt/HIF-1 pathway plays a key role in the EMT of HepG2 cells under hypoxic conditions, inhibiting the expression of E-cadherin and promoting the expression of N-cadherin and vimentin. However, the changes of N-cadherin expression in gene knockout/knockdown, ncRNA silence group were not statistically significant, indicating that N-cadherin may be regulated by multiple genes rather than single factor. Medicine and microenvironmental intervention could acts on multiple regulatory factors, and then affect the expression of N-cadherin. No single gene has proven to cause significant changes on N-cadherin. It could be also related to the following aspects: (1) the amount of research into the three kinds of the group were less (2-3); (2) part of the included trials [55, 75–76] did not report the dose and duration of the intervention, which may also be one of the reasons for greater heterogeneity and inconsistency among the studies; (3) it may be that the types of genes and ncRNAs involved in the included experiments were not consistent, as well as the complex signaling pathways such as TGF- $\beta$ 1, Wnt/ $\beta$ -catenin, EGFR, and NF- $\kappa$ B ultimately led to significant differences in the efficacy of the intervention and resulted in greater heterogeneity among the included studies. Therefore, we suggest the following: (1) when researchers select medicine, genetic intervention, and tumor microenvironment, and overexpression of ncRNA to influence the EMT effect on HepG2 cells, N-cadherin can be used as a secondary marker; (2) More studies involving the expression of N-cadherin regulated by gene knockout/knockdown and ncRNA silence are needed to establish the research system of gene/ncRNA-N-cadherin-signaling pathway to reduce the heterogeneity and improve the conversion and utilization.

We also found that medicine, pro-EMT genetic intervention, gene knockout/knockdown, anti-EMT ncRNA overexpression/silencing, and hypoxia significantly affects Vimentin. Expression of Vimentin increases as epithelial cells transform to mesenchymal cells during the EMT process [91]. Liu et al. [94] showed that Vimentin participates in the EMT of cancer cells by mediating cytoskeletal tissue and local adhesion maturation. Vimentin is upregulated in HCC and then induced in the EMT process [92]. Dan et al. [93] found that Vimentin acetylation is involved in SIRT5-mediated migration in liver cancer. Most antitumor drugs can inhibit the growth and invasion of tumor cells by directly or indirectly inhibiting Vimentin. Satelli A et al [94] found that a Vimentin-binding mini-peptide can bind to Vimentin, target, and interact with Vimentin to interfere with various signaling pathways such as Erk, AKT1, Axl and PI3K and then cell functions. Liu et al. [27] also reported that Fucoindan inhibited the expression of vimentin by inhibiting the

activity of the PI3K/AKT signaling pathway. However, interventions for anti-EMT genetic intervention, tumor microenvironments other than hypoxia, pro-EMT ncRNA overexpression/silencing does not significantly change Vimentin expression. In conclusion, Because of the limitation in the number of included studies (1-2), there is not enough data to support the evaluation effect of Vimentin on the above three interventions. In summary, we believe that the following: (1) Vimentin could be a valuable marker for interventions of medicine, pro-EMT gene overexpression/knockdown/knockout, hypoxia, anti-EMT ncRNA overexpression/silencing act on HepG2 cells to affect the EMT; (2) Future studies should pay more attention to the effects of intervention for tumor microenvironments other than hypoxia, pro-EMT ncRNA overexpression/silencing needs further investigation.

In terms of Snail, interventions of tumor microenvironments other than hypoxia, anti-EMT gene overexpression, gene knockdown/knockdown, anti-EMT ncRNA overexpression, and ncRNA silencing induced significant changes of Snail expression. Snail family of proteins is one of the most important EMT downstream signaling pathway transcription factors. It's a key inducer of EMT, which can regulate invasion and migration [95]. The possible reasons are as follows: During EMT, its downstream signaling pathway transcription factors (EMT-TFS) are activated, mainly including zinc finger transcription factors family (Snail1 and Snail2), Twist family (Twist1 and Twist2), and ZEB family related to zinc finger E-box (ZEB1 and ZEB2), etc. Among them, the Snail family of proteins is a key inducer of EMT, which can regulate invasion and migration [95]. Its main role is to directly binds to the E-Box sequence located in the promoter region of the E-cadherin gene and inhibit its transcription [96], hence promoting EMT. There are many studies about the relationship between the Snail pathway and EMT [97–98], but few studies evaluated its potential as EMT biomarker. (Only 21 articles were included in this paper) Therefore, we believe that Snail could be used as a reference marker in future studies to further investigation are needed to explore its value under various interventions.

Our strength is that we included all different kinds of interventions and performed subgroup analysis to reduce the effects of heterogeneity between studies. The limitation is the heterogeneity of in vitro study, intervention dose and processing time, cell source, culture medium, etc. 65% of the included studies did not provide the concentration of the intervention. That's why SMD was used in the pooled analysis of the data. In addition, the different sources, culture details and intervention time of HepG2 cells in the included experiments all had a certain effect on the subsequent analysis. Therefore, it is suggested that the origin, culture, and intervention time of HepG2 cells should be clarified and unified in relevant studies to could help reduce the heterogeneity among studies. Moreover, more attention should be paid to standardization and adequate reporting of the dose and unit of interventions and determining cell viability in in vitro experimental studies so as to further improve the transparency of cell experimental research. This would eventually improve the utilization value of basic research and provide a reliable theoretical basis for the transformation from basic research to clinical research.

In summary, biomarkers work differently under various intervention in HepG2 EMT model. E-cadherin, N-cadherin, and Vimentin respond well to medicine intervention. E-cadherin work well under genetic intervention. E-cadherin and N-cadherin reflects tumor microenvironment intervention. Under ncRNA

intervention, the expression of E-cadherin, change significantly. Moreover, it is necessary to further standardize the implementation of cell culture and intervention measures in vitro, especially the standardized procedure for the time and dose of various interventions to improve the transparency of the whole process of in vitro cell research and promote its transformation and utilization value.

## Conclusions

Some medicine, some gene, microenvironment and some ncRNA can effectively induce/inhibit EMT process. E-cadherin, N-cadherin, Vimentin and Snail are effective biomarkers during this process. They respond differently to different intervention. Therefore, different biomarkers should be chosen under different intervention based on their performance.

## Abbreviations

EMT: epithelial-mesenchymal transition; CNKI: China National Knowledge Infrastructure; CBM: China Biology Medicine disc; ncRNA: non-coding RNA; USP5: ubiquitin-specific protease 5

## Declarations

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### Author Contributions

**Jing Yan:** Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Software (lead); Validation (equal); Writing- original draft (lead). **Shuli Zou:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Writing- review & editing (equal). **Bei Xie:** Data curation (supporting); Investigation (supporting); Methodology (supporting). **Ye Tian:** Formal analysis (supporting); Investigation (supporting). **Zhiheng Peng:** Formal analysis (supporting); Investigation (supporting). **Zhuan Liu:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting). **Bin Ma:** Conceptualization (equal); Methodology (equal); Validation (lead); Writing- review & editing (equal). **Linjing Li:** Conceptualization (equal); Validation (equal); Writing- review & editing(lead).

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### **Availability of supporting data**

Not applicable.

### **Declarations**

### **Ethics approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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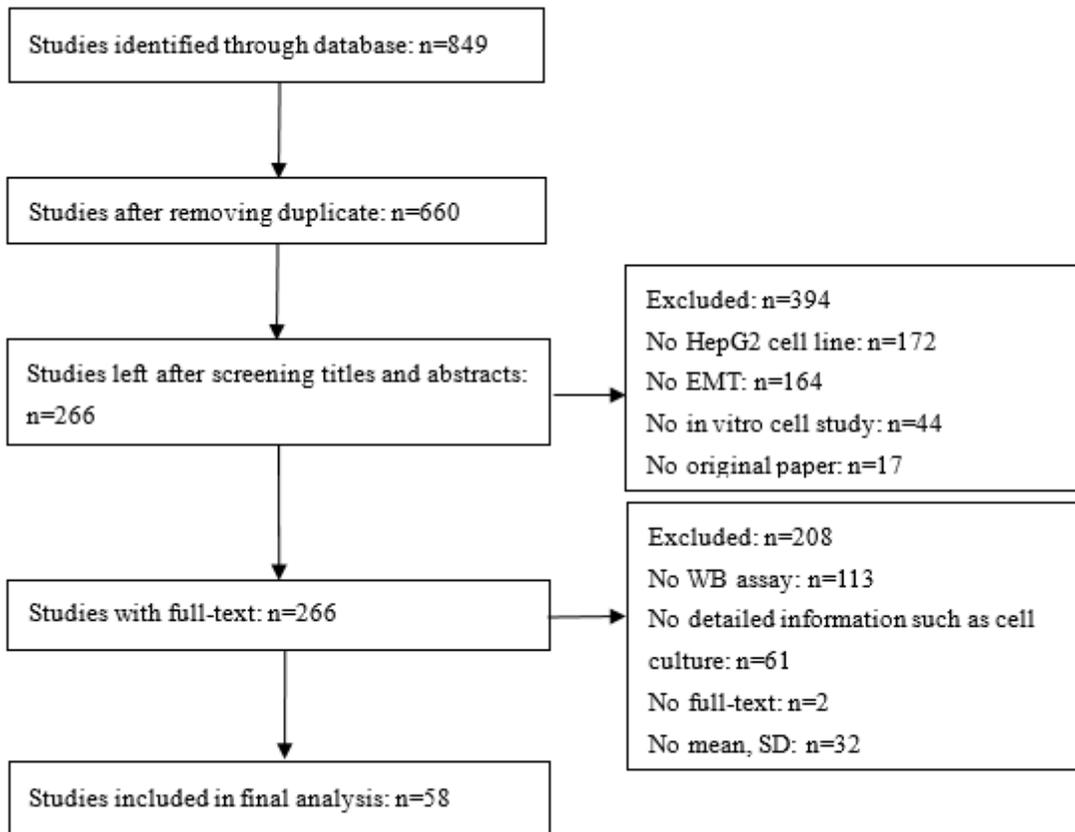
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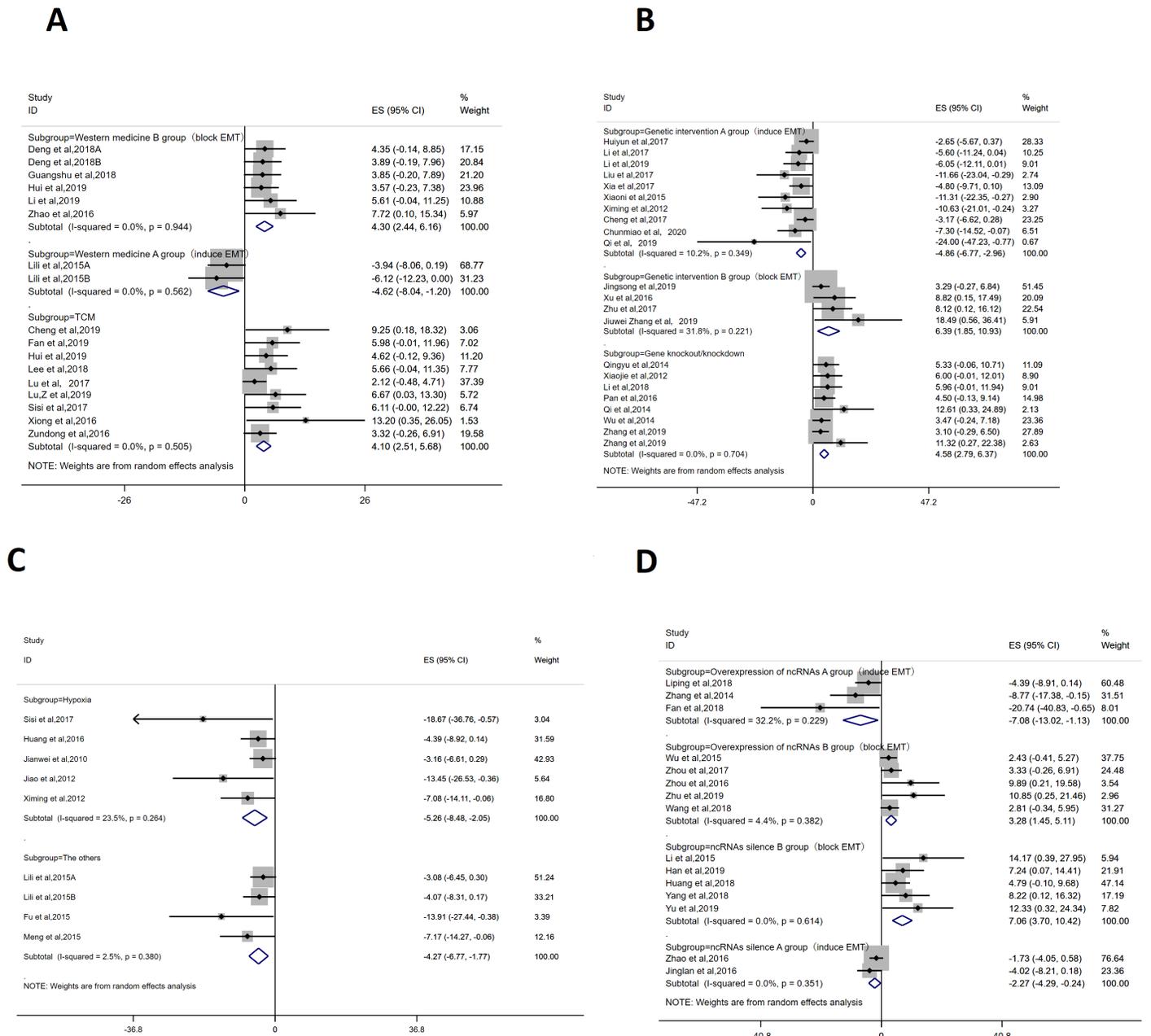
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## Figures



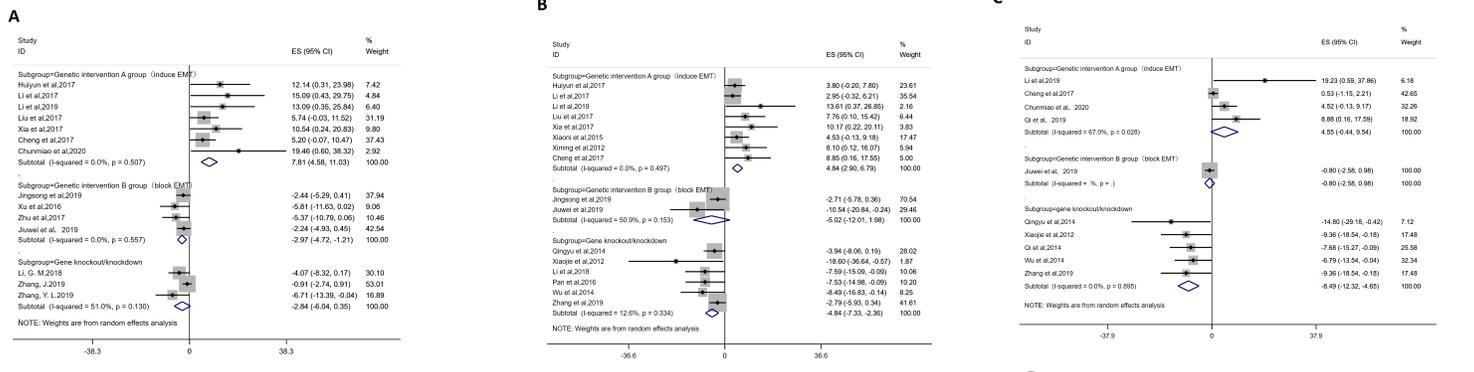
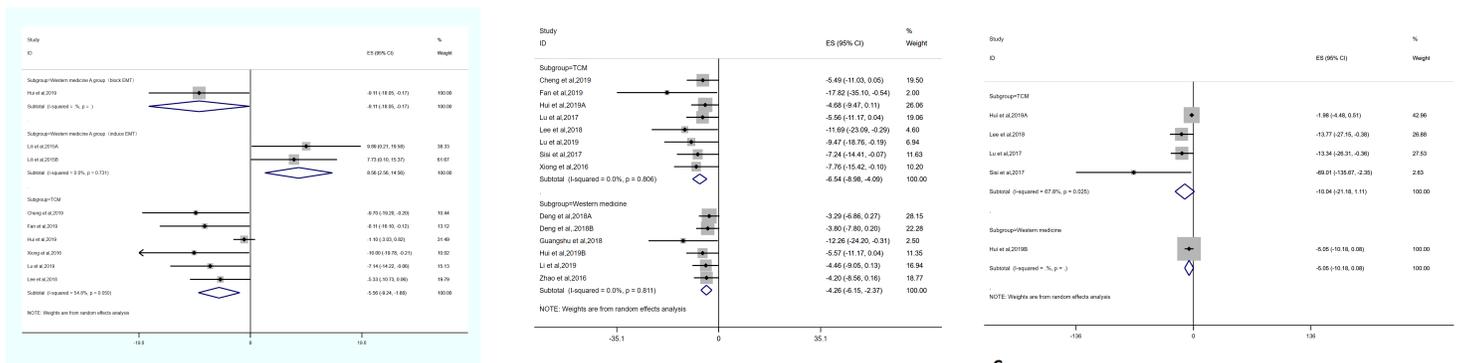
**Figure 1**

Flow chart of the literature search.



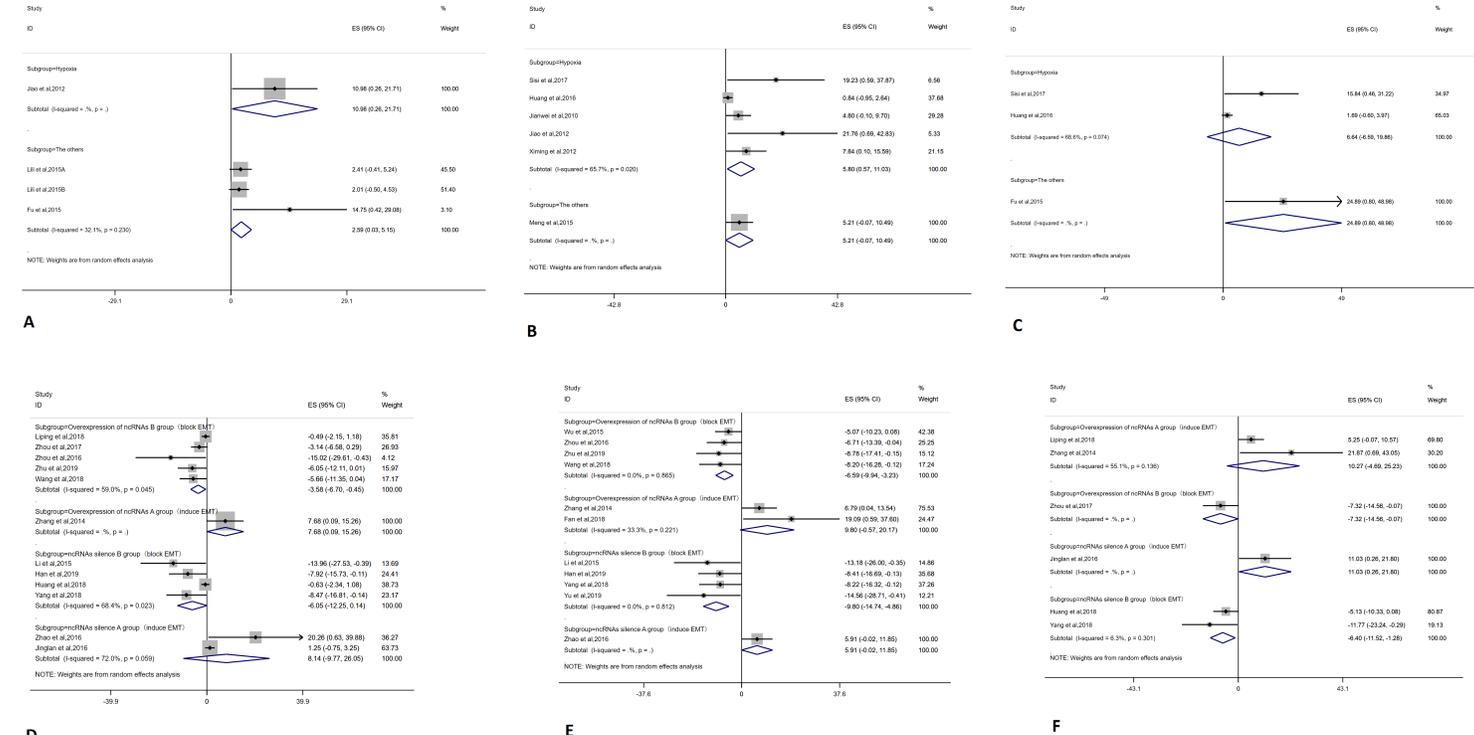
**Figure 2**

Forest plot for the expression level of E-cadherin under four interventions. A, Drug. B, Gene. C, Microenvironment. D, ncRNA.



**Figure 3**

Forest plot for the expression level of N-cadherin, Vimentin, and Snail under drug and genetic intervention. A and D, Forest plot for the expression level of N-cadherin under drug and genetic intervention. B and E, Forest plot for the expression level of Vimentin under drug and genetic intervention. C and F, Forest plot for the expression level of Snail under drug and genetic intervention.



## Figure 4

Forest plot for the expression level of N-cadherin, Vimentin, and Snail under Microenvironmental and NcRNA intervention. A and D, Forest plot for the expression level of N-cadherin under Microenvironmental and NcRNA intervention. B and E, Forest plot for the expression level of Vimentin under Microenvironmental and NcRNA intervention. C and F, Forest plot for the expression level of Snail under Microenvironmental and NcRNA intervention.

## Supplementary Files

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