

# Recent progress on the effects of microRNAs and natural products on tumor epithelial–mesenchymal transition

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**Abstract:** Epithelial–mesenchymal transition (EMT) is a biological process of phenotypic transition of epithelial cells that can promote physiological development as well as tissue healing and repair. In recent years, cancer researchers have noted that EMT is closely related to the occurrence and development of tumors. When tumor cells undergo EMT, they can develop enhanced migration and local tissue invasion abilities, which can lead to metastatic growth. Nevertheless, two researches in *NATURE* deny its necessity in specific tumors and that is discussed in this review. The degree of EMT and the detection of EMT-associated marker molecules can also be used to judge the risk of metastasis and to evaluate patients' prognosis. MicroRNAs (miRNAs) are noncoding small RNAs, which can inhibit gene expression and protein translation through specific binding with the 3' untranslated region of mRNA. In this review, we summarize the miRNAs that are reported to influence EMT through transcription factors such as ZEB, SNAIL, and TWIST, as well as some natural products that regulate EMT in tumors. Moreover, mutual inhibition occurs between some transcription factors and miRNAs, and these effects appear to occur in a complex regulatory network. Thus, understanding the role of miRNAs in EMT and tumor growth may lead to new treatments for malignancies. Natural products can also be combined with conventional chemotherapy to enhance curative effects.

**Keywords:** epithelial–mesenchymal transition, miRNA, tumor, natural products, ZEB, SNAIL

## Introduction

As a biological process, epithelial–mesenchymal transition (EMT) plays important roles in tissue growth, remodeling, and repair of various pathological injuries. EMT is also a significant factor in determining the malignancy of a tumor<sup>1</sup> for an important consideration given that metastatic growth is the direct cause of death in many cancer patients. Consequently, the relationship between EMT and tumors is a significant focus in cancer research. The three main features of EMT on which research is focused include 1) intracellular cell protein levels, including decreased expression of epithelial marker proteins such as E-cadherin (E-CAD),  $\beta$ -catenin, and cytokeratin; it also focuses on the increased expression of mesenchymal marker proteins such as N-cadherin (N-CAD) and vimentin (VIM). 2) Cell phenotypes and biological behaviors, such that epithelial cells lose their polarity and connection with the basal membrane, and then transform into mesenchymal phenotypes with higher migration and invasive tendencies, antiapoptotic features, and production of an extracellular matrix. When EMT occurs in tumors, it tends to increase the migratory ability of the cells and give rise to malignancies. 3) In epigenetics, the occurrence of EMT is associated with epigenetic reprogramming of the genome, involving DNA methylation and post-translational

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modifications of histone.<sup>2</sup> These changes can promote the expression of stromal genes or inhibit the expression of genes related to the epithelial phenotype.

The microRNAs (miRNAs) are 22–24-nucleotides (nts) endogenous, noncoding small RNAs that play a variety of regulatory roles in cells. Most miRNAs regulate target mRNA through base-pairing between seed sequences, which range from nt positions 2–8, located at the 5' end of the miRNA and target the mRNA 3' untranslated region (UTR).<sup>3</sup> Because they have a smaller number of nts in a complementary sequence and relatively low requirements for matching, a single miRNA can target a variety of mRNAs, with many individual mRNA sequences being regulated by various miRNAs.<sup>4</sup> In recent years, many miRNAs that play a regulatory role in EMT have been identified, and most of those that inhibit EMT have a common feature: their expression is decreased in tumor cells, thus promoting occurrence of EMT. However, miRNA re-expression will inhibit that process, mainly by reducing levels of EMT-related transcription factors such as ZEB, SNAIL, TWIST, and SLUG. However, some miRNAs promote EMT through other mechanisms. In addition to miRNAs, some natural products are currently known to regulate EMT or, alternatively, tumor inhibition. The structural diversity and highly selective mechanisms of action of these natural products suggest that they may have great value for the development of antineoplastic drugs.<sup>5</sup>

In this paper, we address the role of EMT in neoplasms, and summarize the effect of miRNAs in the regulation of EMT. We will discuss the function and mechanism of miRNAs and natural products in detail.

## EMT and tumor metastasis

In the traditional view, tumors were considered to be a homogeneous proliferation of malignant cells, but more recent studies have shown that solid neoplasms are complex tissues composed of multiple cell types, with considerable intratumoral heterogeneity. The difference between cancer stem cells and non-stem cells is a major source of this heterogeneity. Apart from showing remarkable genetic and phenotypic variabilities, cancer stem cells maintain plasticity between epithelial and mesenchymal cell types under the regulation of their microenvironment, which influences tumor metastasis.<sup>6</sup> EMT has been identified and described as the underlying process for tumor metastasis in the last two decades.<sup>7</sup> Metastases occur through a series of steps: local infiltration, entry of cancer cells into blood vessels, transport in the circulatory system, and finally, exuding from blood vessels, and achieving colonization.<sup>8</sup> The neoplastic cells then

disseminate from the primary site and form secondary tumors at other distant sites. Metastasis is a significant hallmark of malignancy, and it seriously affects the patient's prognosis, treatment plan, and quality of life.

## EMT and local infiltration of tumor

EMT is the first step in the cascade of steps involved in tumor invasion and metastasis. The original malignant epithelial cells lose intercellular junctions and lumen–basement polarity and thus develop the capacity to migrate, cross basement membranes, and invade blood vessels. After entering their local blood vessels, these cells can give rise to clusters of circulating tumor cells (CTCs) in the bloodstream. At some point, they can leave the blood vessels, escape the circulation, and form micrometastases at distant organs.<sup>9</sup> For example, for tissue invasion and metastasis, colorectal cancer cells (CRCs) at the tumor–normal tissue interface frequently undergo EMT, losing epithelial marker proteins and acquiring mesenchymal ones. Then, they lose cell-to-cell adhesion, and finally become invasive. We have known that the progression of colorectal cancer could be promoted by the pro-inflammatory factor interleukin 6 (IL-6) in the tumor microenvironment. Liu et al<sup>10</sup> suggested that an aberrant IL-6/STAT3/Fra-1 signaling pathway promoted cellular invasion and metastasis in colorectal cancer patients through EMT. They found that the ectopic expression of Fra-1 significantly reversed the STAT3-knockdown effect, and enhanced CRC cell aggressiveness, by promoting the expression of EMT-related factors ZEB1, SNAIL, SLUG, matrix metalloproteinases-2 (MMP-2), and MMP-9. Furthermore, it has been reported that breast cancer cell line py2t from the MMTV-PyMT transgenic mouse can be induced to undergo reversible EMT by cell transforming growth factor- $\beta$  (TGF- $\beta$ ) treatment in cell model systems, accompanied by downregulation of E-CAD, the gain of stromal cell markers, and expression of EMT transcription factors. At the same time, the motility and aggressiveness of the single cancer cells were elevated.<sup>11</sup>

## The relationship between EMT and tumor cells exuding from vessel lumens

EMT is often accompanied with the dephosphorylation of certain proteins like  $\alpha$ -tubulin. This mediates the formation of tubulin microantenna, which induces CTCs of mesenchymal phenotypes to attach to the endothelial layer, and further migrate from blood vessels to colonize distant loci. Furthermore, GLU microprotein (a kind of  $\alpha$ -tubulin) increases the life span of tumor cells in blood vessels and promotes cells exuding from blood vessels.

## EMT and tumor metastasis

In the process of tumor colonization, cancer cells usually regain epithelial features via reversing EMT (or mesenchymal–epithelial transformation – [MET]) to form a secondary tumor and large patchy metastases, resulting in the completion of the cascade reaction of invasion and metastasis. Although metastatic tumors show the typical characteristics of EMT in both their original tumor cells and the CTC clusters, the distant metastases of most epithelial carcinomas usually have epithelial features. MET plays an important role in assisting disseminated tumor cells to form large metastatic foci in distant organs.<sup>6</sup> It has been reported that the invasive ability and transepithelial migration of E-CAD-negative prostate cancer cells is greater than that of E-CAD-positive prostate cancer cells.<sup>12</sup>

## EMT score and tumor evaluation

Interestingly, it has been reported that conversion between epithelial and mesenchymal phenotypes is not necessarily complete. Neoplastic cells can acquire a mixed epithelial–mesenchymal phenotype that is called incomplete or partial EMT.<sup>9</sup> The level of EMT in ovarian cancer, breast cancer, and colorectal cancer has been assessed by measuring the transcription level of tumor-specific EMT markers, and an EMT score was evaluated and used to analyze the interaction between EMT and tumor progression. The results showed that there was a good correlation between the EMT score and previously identified tumor-specific EMT markers in ovarian cancer and colorectal cancer, but not in breast cancer.<sup>13</sup> The EMT score can be used to diagnose and evaluate the development of tumors as well as their drug resistance.

## Unconventional discovery: EMT is not required for tumor metastasis

It is worth noting that a study in *NATURE* has put forward different ideas and challenged the existing hypotheses about the role of EMT in tumor metastasis. They invented a mesenchymal-specific, Cre-mediated fluorescent marker switch strategy and established a triple transgenic mouse model (Tri-PyMT, MMTV-PyMT/Rosa26-RFP-GFP/Fsp1-Cre), which facilitated breast-to-lung metastasis. As a result, they found that tumor cells maintained their original epithelial phenotype in the primary tumor and they did not activate the mesenchymal-specific FSP1 promoter, but retained their epithelial phenotype during metastasis. More importantly, tumor cells in metastatic lesions were not manifested to have experienced EMT process during metastasis either. Then they injected miR-200-overexpressing Tri-PyMT cells in vivo.

To much depression, inhibition of EMT by miR-200 overexpression did not impair the ability of tumor cells to form distant lung metastases. Therefore, they believe that EMT is not required for lung metastasis in breast cancer.<sup>14</sup> At the same time, there is another study in *NATURE* that points out that EMT is not essential in the metastasis of pancreatic cancer.<sup>15</sup> Different from the above article, they confirmed their conclusion in the reverse perspective. They would like to check whether EMT inhibition will repress tumor metastasis. Finally, it was found that knocking out twist, snail genes did not inhibit tumor metastasis in vivo at all.

Both research methods are new and reasonable, and they put forward different conclusions from past views. There may be two reasons accounting for this disparity. First, most of the studies about EMT are in vitro experiments, while there is a gap between them and the actual situation of tumor development in vivo. In addition, what both studies point out is that the tumor does not undergo EMT during metastasis, which is not contradictory to previous verdict that EMT promotes tumor metastasis. The EMT process leads to a decrease in cell adhesion, endowing tumor cells features contributing to easier migration. There is no doubt that EMT promotes tumor metastasis, but whether it happens during the complicated process in all metastatic situations is uncertain. That is understandable. After all, tumor metastasis involves so many known and undiscovered mechanisms. What is more, these two researches are studied in mouse, remaining the argument of the potential role of EMT in human cancer metastasis.

## Regulation of miRNA on EMT in tumors

### Inhibition of EMT via miRNA/ZEB pathway

Oba et al<sup>16</sup> revealed 82 candidate miRNAs that have possible interaction with ZEB-2 through analyzing complementary sequences of miRNA and ZEB-2 mRNA 3' UTR. Then, each miRNA and a luciferase reporter vector containing the 3' UTR region of the ZEB-2 mRNA were cotransfected into NIH3T3 cells. They found that miR-200, miR-666-5p, and miR-183 had significant effects, decreasing the activity of ZEB-2 more than 20% compared to the control group. The ZEB-2 protein inhibits E-CAD expression, allowing these miRNAs to inhibit tumor EMT. This has been confirmed in their experiments. There are three kinds of stable state of miR-200/ZEB: high miR-200/low ZEB; low miR-200/high ZEB; and moderate miR-200/ZEB, which represent the epithelial, mesenchymal, and mixed states, respectively.<sup>17</sup>

### p53/miRNA/ZEB

It is summarized in a review that miRNA molecules regulated by P-53 include miR-192 family (miR-192, miR-194, miR-215), miR-107, miR-145, miR-200s, and miR-34s (+7).<sup>18</sup> Through a comparative study of (CD24<sup>+</sup>CD44<sup>+</sup>, stem) HMECS and (non-CD24<sup>+</sup>CD44<sup>+</sup>, non-stem) MCF12A cells, it was found that miR-183 and miR-200c were significantly downregulated in stem cells (>2 times). Further studies have revealed that P53 can act on the upstream transcription initiation region of both miR-183 and miR-200c. Chromatin immunoprecipitation analysis confirmed that P53 could bind specifically with their promoter-specific fragment and promote their expression. Besides, P53 was able to regulate EMT in MCF12A cells through miR-200c. P53 knockdown decreased the expression of E-CAD, while N-cadherin, VIM, and ZEB1 were increased. The exogenous expression of P53 gene could reverse the TGF- $\beta$ -induced EMT.<sup>19</sup> This study links the most important genetic alteration in human cancer to the emerging fields of EMT and cancer stem cells, which are of importance for metastasis.<sup>20</sup> In ovarian cancer cells, the luciferase reporter assay revealed that mutations in P53 binding region in the miR-200b/a/429 and miR-200c/141 promoters reduced luciferase activity by 40% ( $P < 0.003$ , *t*-test).<sup>21</sup> Another relevant research indicated overexpression of P53 upregulated miR-145 and inhibited EMT process, with ZEB2, fibronectin, and VIM expression increased but E-CAD decreased in prostate cancer, and inhibit bone metastasis in vivo.<sup>22</sup> In prostate cancer, double-negative feedback loop between ZEB2 and miR-145 contributes to PCa progression and metastasis, and might have therapeutic relevance.<sup>23</sup>

The expression of miR-192 family is also related to upregulation of P53 protein expression. P53-regulated miR-192 family members also repress ZEB2 expression.<sup>24</sup> Overexpression of these molecules can inhibit the expression of MDM2 (murine double minute 2) at mRNA and protein levels, thus reducing the inhibitory effect of MDM2 on P53 gene. This autoregulatory loop is a positive feedback loop with the feature of double-negative regulation.<sup>25</sup>

### The role of miRNA/ZEB in head and neck squamous cell carcinoma

EMT in head and neck squamous cell carcinoma (HNSCC) can be inhibited by miR-138, which reduces local invasion and metastases. It mainly involves three mechanisms: 1) direct targeting of VIM inhibits mRNA translation, 2) targeting of ZEB2 and SNAI2 genes to regulate the E-CAD transcription, and 3) directly acting on histone lysine methyltransferase EZH2 (or enhancer of zeste homolog 2),

which can inhibit the transcription of downstream E-CAD genes. These points help to promote the inhibitory effect of miR-138 on EMT.<sup>26</sup>

### The role of miRNA/ZEB in breast cancer

MiR-200b-3p/5p was found to upregulate MET-related genes and epithelial-cadherin gene (Cadherin-1) and attenuate cell migration in triple-negative breast cancer (TNBC). Interestingly, there was no obvious decrease of ZEB, and it decreased neoplastic invasion by inhibiting the Rho signaling pathway rather than the classical pathway, miR-200/ZEB/EMT, in this process.<sup>27</sup> Kong et al<sup>28</sup> proposed that 53BP1 was a tumor suppressor gene in breast cancer, and that its overexpression in MDA-MB-231 cells increased E-CAD content, accompanied by a significant decrease in ZEB1 and increased expression of miR-429 and miR-200b. Therefore, 53BP1 may inhibit EMT of breast cancer cells and metastasis in vitro and in vivo and through the miR-200/ZEB/EMT pathway. Nevertheless, some studies have put forward opposite conclusion. Low level of miR-200 expression was detected in claudin-low breast cancer (CLBC). Re-expression of miR-200c in vitro did not change the expression of ZEB1/2 significantly. Invasion chamber assay showed little diminishment in tumor cell invasion either.<sup>20</sup> What is more, another study has shown that miR-141/200 re-expression increased the ability to invade and migrate in human CLBC cell line MDA-MB-231. So the universality of miRNA200s function remains to be further studied.<sup>29</sup>

In animal experiments, MDA-MB-231 cells (human breast cancer cells) were implanted into the left ventricles of mice,<sup>30</sup> with bone metastases occurring 8–12 weeks later and bone metastasis lesions formed. The differential expression of 118 kinds of miRNA (>2 times) was found by analyzing primary tumor cells (231-P) and metastatic tumor cells (231-B). Sixteen kinds of miRNA expression were upregulated; conversely, another 102 kinds of miRNA were downregulated. Specifically, miR-429 was significantly downregulated in 231-B cells. That was consistent with the results of previous studies: miR-429 is known to inhibit the invasion and metastasis of various tumors. Further studies found that overexpression of miR-429 in 231-B cells could significantly reduce their invasive and metastatic activity. Western blot studies showed that ZEB1 and Crk-like protein were significantly reduced by miR-429, and EMT was repressed as well. In addition, miR-486-5P was also found to be significantly downregulated in the 231-B cells, which has been reported recently to inhibit lung cancer metastasis. Also, miR-147 has also been shown to



inhibit EMT induced by TGF- $\beta$  in A549 lung cancer cells via acting on ZEB.<sup>31</sup>

### The role of miRNA/ZEB in lung cancer

A new study discovered Foxf2 (forkhead box F2, a member of transcriptional regulators) as the new targeting molecule of miR-200b/c, which mediated EMT and promoted invasion and migration of lung cancer cells in non-small-cell lung cancer (NSCLC).<sup>32</sup> The miR-155 and miR-200c expressions were downregulated in gemcitabine-resistant (GR) cells (HCC827GR), with their target molecules Smad and ZEB1 increased at the same time. EMT process was detected as well.<sup>33</sup> Decitabine is able to reverse TGF- $\beta$ -mediated EMT through inhibition of miR-200 promoter methylation and repression of miR-200/ZEB axis in NSCLC.<sup>34</sup> Chen et al<sup>35</sup> concluded that GSK-3 was involved in EMT through snail inhibition. In docetaxel-resistant lung adenocarcinoma cell model, miR-451 re-expression inhibited extracellular-signal-regulated kinase (ERK)-dependent GSK-3 phosphorylation, which represented its inactivation via acting on C-MYC, so as to repress snail-mediated EMT.

### The role of miRNA/ZEB in pancreatic cancer

In the mechanisms of EMT, ZEB interacts with the C-terminal-binding protein (CtBP) to inhibit expression of miR-200s. Both CtBP and ZEB have interaction sites on the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429). Conversely, miR-200s regulates EMT through ZEB inhibition. Sass et al<sup>36</sup> found that overexpression of miR-141 and miR-200c led to a reduced expression of CtBP and ZEB1 in pancreatic cancer cells, and then inhibited the occurrence of EMT. Thus, miR-200a plays the same role in pancreatic cancer cells through ZEB1.<sup>37</sup> Moreover, miR-655 can also inhibit invasion and migration of pancreatic and breast cancer cells by acting on ZEB.<sup>38</sup>

### The role of miRNA/ZEB in bladder cancer

It was found in two bladder cancer cell lines that invasion by CRL1749 cells was inhibited by enhancing the expression of miR-141 or miR-200b, but opposite results occurred with HTB9 cells after downregulating miR-141 or miR-200c. Moreover, an increase of miRNA expression in the CRL1749 cells was accompanied by a significant decrease in the activity of MMP-2 and MMP-9, while the opposite was found in HTB9 cells. Because MMPs could promote tumor invasion and metastasis, we anticipated that both MMP and EMT inhibition were significant mechanisms by which miR-200s repressed tumor metastasis.<sup>39</sup>

## Inhibiting EMT via miRNA/SNAIL pathway

The SNAIL/miR-34 family (miR-34a, miR-34b, and miR-34c) forms another system for epithelial and mesenchyme morphogenesis.<sup>40</sup> The miR-34 inhibits their translation through base-pairing in conserved region of SNAIL1, SNAIL2, and ZEB1 3' UTR, and ultimately inhibits the occurrence of EMT. Conversely, SNAIL can directly inhibit the miR-34 promoter to reduce the expression of miR-34. Actually, miR-34 expression is usually downregulated while SNAIL expression increased in tumor cells, which further inhibits the expression of miR-34. Such bidirectional negative regulation mechanism, making each molecule linked more closely and more efficient, has better regulating effect on tumor cell EMT. Noticeably, miR-34 may repress EMT via other mechanism instead of SNAIL. It was confirmed that miR-34a acted on SNAIL to regulate EMT in breast cancer and lung cancer cells, but there was no such regulation in prostate cancer PCa cells, replaced by significant down-regulation of lymphoid enhancer-binding factor 1 (LEF-1). Then the expression of E-CAD was increased, while N-CAD expression decreased.<sup>41</sup> Therefore, regulating the expression or activity of miR-34a or LEF-1 in prostate cancer cells may play a positive role in cancer treatment.

### p53/miRNA/SNAIL

There was a study pointing out that when P53 gene got mutation or lost its function, the miRNA-34 level was reduced. It could not inhibit SNAIL1 at protein level as usual, which promoted tumor cell EMT. Although P53 gene could directly affect cell-cycle regulation, cell apoptosis, and DNA repair pathways, the EMT and metastasis process induced by P53 inactivation completely depended on the expression of SNAIL1, which is common and important in terminal cancer.<sup>42</sup>

### miRNA/SNAIL/ZNF281

There is a molecule closely related to SNAIL/miR-34/EMT regulatory system – ZNF281. ZNF281 is a transcription factor that mediates transcriptional activation or inhibition, regulating tumor cells proliferation, differentiation, and apoptosis. A study about colorectal cancer showed that SNAIL could promote the expression of ZNF281, which is positively correlated with mesenchymal markers such as VIM in breast cancer SKBR3 cells, pancreatic cancer MiaPaCa2 cells, and colorectal carcinoma DLD-1 cells. When they used small interfering RNA (siRNA) to interfere with ZNF281 expression, the EMT induced by SNAIL was

significantly weakened.<sup>43</sup> C-MYC inducing the occurrence of EMT also depends on ZNF281, and C-MYC cannot bind with ZNF281 promoter, but activates downstream EMT via promoting the expression of SNAIL. It maintains mesenchyme phenotype in CRC cell lines, and promotes tumor invasion and metastasis.<sup>43</sup>

### miRNA/SNAIL/G9a

G9a, as a kind of histone H3 lysine 9 methyltransferase, is a downstream molecule of SNAIL, which can form a complex with DNA methyltransferase under the recruitment of SNAIL, and bring methylation of E-CAD promoter to inhibit its expression. G9a knockdown reduced cell invasion and metastasis in vitro and lung metastasis in vivo of CLBC.<sup>44</sup> Liu et al<sup>45</sup> have also confirmed that G9a promotes EMT and lymph node metastasis in head and neck cancer cells through such mechanism.

### The role of miRNA/SNAIL in liver cancer

The expression of miR-451 is downregulated in liver cancer cells, which mediates the overexpression of C-MYC, thus activating the Erk1/2 signaling pathway. Then this pathway regulates the GSK-3 $\beta$ /SNAIL/E-CAD cascade reaction to promote EMT. In addition, miR-451 also inhibits cellular growth and promotes apoptosis. Therefore, it is likely that a new method for treatment of metastatic liver cancer can be developed by regulating the miR-451/C-MYC/Erk1 and 2 axis.<sup>46</sup> Injecting miR-449a into hepatocellular carcinoma (HCC) cells can inhibit its formation of foci of invasion and metastasis in vitro.<sup>47</sup> It combined with the 3' UTR of FOS (FBJ murine osteosarcoma viral oncogene homolog) and MET (pro-oncogene, receptor tyrosine kinase) to reduce their expression, thereby inhibiting the downstream signaling pathways, including Protein Kinase B (AKT) phosphorylation and the GSK-3 $\alpha/\beta$  activity. Then it decreased nuclear SNAIL accumulation and raised the E-CAD level. While the miR-29b/SNAIL axis inhibited EMT in prostate cancer, Yan et al<sup>48</sup> found that miR-29b inhibited MMP-2 expression, which contributed to EMT as well in HCC. It is also recognized that fucoidan upregulated miR-29b expression in HCC, in addition to TGF- $\beta$  receptors and SMAD signaling inhibition. All of these effects contributed to cooperative inhibition of the occurrence of EMT in HCC.

The LET-7 family consists of 12 miRNAs, which are also regulators of cell differentiation, directly taking effect on transcription factors related to oncogene activity or SNAIL expression to inhibit tumor growth. LET-7 expression was decreased in various tumors including lung cancer, colon

cancer, and ovarian cancer.<sup>40</sup> Chen et al<sup>49</sup> found that LET-7g expression was the lowest, and transfection of the LET-7g gene in vitro significantly inhibited the malignant invasion of HCC cells. Mechanistic studies showed that LET-7g re-expression inhibited EMT in HCC cells through blocking the K-Ras/HMGA2A/SNAIL axis. Liver cancer cell line HCCLM3 was transplanted into nude mice to form a tumor. Then LET-7g plasmids were injected into the tumor, after which its volume was significantly reduced. Therefore, LET-7g may serve as an ideal treatment for early HCC patients. In NSCLC and liver cancer, miR-30a promoted E-CAD expression by acting on SNAIL1.<sup>50</sup> However, miR-30c was directly targeted to VIM and fibronectin to inhibit tumor metastasis in lung cancer and hepatoma. The fragile histidine triad gene product (FHIT) could upregulate miR-30c, and thus FHIT may provide an effective intervention for early lung cancer and hepatoma.<sup>51</sup>

### The role of miRNA/SNAIL in gastrointestinal tumor

The expression of miR-22 was significantly reduced in clinical gastrointestinal tumors and was tightly associated with poor prognosis. The introduction of miR-22 significantly inhibited gastroenteric cancer cell growth, migration, and invasion in vitro. It targeted mRNA of SNAIL and MMP14 molecules to inhibit tumor progression; meanwhile, experiments in vivo have confirmed that overexpression of miR-22 inhibited tumor growth, peritoneal dissemination, and lung metastasis.<sup>52</sup>

### The role of miRNA/SNAIL in prostatic cancer

The miR-29b and miR-30 could also inhibit the expression of SNAIL. In prostate cancer cells, overexpression of miR-29 induced complete mesenchymal epithelial transition, which greatly depressed the invasiveness of prostate cancer cells.<sup>53</sup>

## Inhibition of EMT via the miRNA/TWIST pathway

### The role of miRNA/TWIST in breast cancer

MiR-300 was an obvious type of miRNA that was downregulated after HNSCC cells, and breast cancer cells had undergone EMT. Transducing miR-300 inhibitor in low-metastatic HN-4 and MCF-7 cells could induce EMT, but miR-300 could reduce TWIST expression in highly metastatic HN-12 and MDA-MB-231 cells. Moreover, overexpression of TWIST could weaken the inhibition of miR-300 on EMT, and the complementary region of miR-300 and TWIST 3' UTR further indicated that miR-300 directly targets TWIST to suppress EMT.<sup>54</sup>

Similarly, miR-129-5p can also inhibit the expression of EMT in breast cancer cells. But miR-129-5p is also one of the transcriptional regulatory targets of TWIST and SNAIL. Gene chip detection using the luciferase reporter gene assay showed that overexpression of TWIST1 or SNAIL decreased miR-129-5p promoter activity, but knockdown of TWIST1 or SNAIL increased it. Therefore, there are inhibitory effects between miR-129-5p and SNAIL. Downregulation of miR-129-5p in tumor cells indicates an increased expression of TWIST and occurrence of EMT, which results in poor prognosis in patients with breast cancer.<sup>55</sup>

The expression of miR-33b in breast cancer cells is also decreased, and its ectopic expression could inhibit the proliferation, invasion, and metastasis of tumor stem cells.<sup>56</sup> Results of initial screening of miR-33b target genes using online databases, quantitative reverse transcription polymerase chain reaction, and luciferase detection indicate that TWIST1, HMGA2, and SNAIL are downstream targets of miR-33b. MiR-155 can downregulate TWIST1, TCF4 (transcription factor 4), VIM-1, and ZEB2 in mouse breast cancer cells. Experiments in vivo show that it does not affect the growth of primary tumor cells, but inhibits EMT.<sup>57</sup>

### The role of miRNA/TWIST in liver cancer

PDGF-D (platelet-derived growth factor D) plays an important role in EMT and drug resistance through the PDGF-D/miR-106a/TWIST pathway in HCCs. Introducing the miR-106a analog into HCC cells decreases TWIST expression and suppresses invasion and metastasis by GR HCC cells. But PDGF-D mainly inhibits the expression of miR-106a. In contrast to prostate cancer cells, which develop EMT with inhibition by miR-200b, there are no changes of miR-200b level in the HCC cells.<sup>58</sup> Inactivation of the PDGF-D/miR-106a/TWIST pathway or direct activation of miR-106a expression may be a novel strategy for the treatment of HCC.

### The role of miRNA/TWIST in prostatic cancer

In in vitro experiments, miR-186-TWIST axis inhibited EMT initiation in prostate cancer (M12 cell line), accompanied by decreased cell invasion and migration abilities. Moreover, Kaplan–Meier survival analysis revealed that patients with high level of miR-186 had a higher survival rate than those with low level of miR-186 ( $P=0.028$ ).<sup>59</sup>

In summary, in different types of tumors, there may be different miRNA molecules that inhibit EMT, and these miRNAs can directly or indirectly regulate multiple target molecules; multiple miRNAs can regulate EMT by the same molecular pathway. The list of miRNAs and their target

molecules and the tumor types that they inhibit to affect the occurrence of EMT, as elucidated in recent years, are provided in Table 1.

## miRNA promoting EMT

Although most of the miRNA molecules such as miR-200 and miR-34 inhibit invasion and metastatic process of neoplastic cells by depressing EMT-related transcription factors, there are still some miRNAs that exert the opposite effect.

### miR-9 promoting EMT

Song et al<sup>63</sup> reported that miR-9 could bind with E-CAD mRNA 3' UTR. A stable transfection of miR-9 on esophageal adenocarcinoma cell lines HKESC1 and KYSE410 decreased E-CAD expression at both RNA and protein levels. It promoted EMT in colon cancer cells as well, and the mechanism is related to the PROX1 (Prospero homeobox 1) gene.<sup>64</sup> In liver cancer, miR-9 promoted EMT invasion and metastasis through KLF17 (Krüppel-like factor 17).<sup>65</sup>

### miR-27a promoting EMT

Xu et al<sup>66</sup> found that SP<sup>Fbxo45</sup> (the atypical E3 ubiquitin ligase complex SKp1-Pam-Fbxo45) could downregulate core transcription factors of EMT, such as ZEB1/2, SNAIL, and TWIST1, through the SPRY domain (ZEB2) and F-box domain (SNAIL1/2, TWIST2), respectively. Additionally, miR-27a could inhibit the expression of Fbxo45 through direct interaction, to maintain the phenotype of EMT. Therefore, inhibiting miR-27a or raising E3 activity may provide new methods to treat cancer and other diseases.<sup>66</sup>

### miR-221/miR-373 promoting EMT in breast cancer

MiR-373 targets the 3' UTR of TXNIP (thioredoxin-interacting protein) mRNA to reduce the expression of TXNIP, and then increases ROS (reactive oxygen species) and activates the HIF-1 $\alpha$  (hypoxia-inducible factor)-TWIST signal axis, which will promote EMT in the end. The TWIST binding with miR-373 promoter sequences could stimulate miR-373 expression and form a positive feedback loop to strengthen these effects. Finally, the presence of miR-373 worsens prognosis in breast cancer patients.<sup>67</sup> Overexpression of miR-221 in tumor cells also promotes EMT.<sup>68</sup> As a proto-oncogene-related miRNA, miR-221 plays an important role in the proliferation and migration of pancreatic cancer, mainly via inhibiting PTEN, P27<sup>kip1</sup>, P57<sup>kip2</sup>, PUMA, and other tumor suppressors.<sup>69</sup> Peptide nucleic acid (PNA) is an analog of DNA, and the sugar-phosphate backbone is replaced by N-(2-aminoethyl) glycine. Piva et al<sup>70</sup> found that experimental

**Table 1** miRNAs known to inhibit EMT

miRNA suppressing EMT	Target	Cancer types	References
miR-145	ZEB2	Prostate cancer	22
miR-192	ZEB2	Multiple myeloma	25
miR-138	ZEB2, SNAIL2, vimentin, EZH2	Head and neck squamous cell carcinoma	26
miR-155	ZEB1, Smad	Lung cancer	33
	TWIST1, TCF4, VIM-1, ZEB2	Breast cancer	57
miR-200			
miR-200a	ZEB1	Pancreatic cancer	37
miR-200b	ZEB, MMP-2/9	Bladder cancer	39
	Rho, Foxf2	Breast cancer, lung cancer	27, 32
miR-200c	ZEB1, CtBP	Pancreatic cancer	36
miR-141	ZEB1, CtBP	Pancreatic cancer	36
	MMP-2/9	Bladder cancer	39
miR-429	ZEB1, CRKL	Breast cancer	30
miR-147	ZEB1	Lung cancer, colorectal cancer	31
miR-451	SNAIL, C-MYC	Lung cancer, hepatocellular carcinoma	35, 46
miR-655	ZEB1, TGFBR2	Esophageal squamous cell carcinoma	38
miR-34			
miR-34a	SNAIL, ZEB1, SLUG	Breast cancer, lung cancer, prostate	40, 42
miR-34b	LEF-1, $\beta$ -catenin, Axin2	cancer, colon cancer	
miR-34c			
miR-29b	SNAIL, MMP-2	Prostate cancer, hepatocellular carcinoma	48, 53
miR-186	TWIST	Prostate cancer	59
miR-23a-3p	E-CAD	Prostate cancer	60
miR-22	SNAIL, MMP14	Gastroenteric tumor	52
miR-449a	MET, FOS	Hepatocellular carcinoma	47
LET-7	SNAIL	Hepatocellular carcinoma	49
miR-30a	SNAIL1	Lung cancer, hepatocellular carcinoma	50
miR-30c	Vimentin, fibronectin	Lung cancer, hepatocellular carcinoma	51
miRNA300	TWIST	Head and neck squamous cell carcinoma, breast cancer	54
miR-129-5p	TWIST, SNAIL	Breast cancer	55
miR-33b	TWIST1, HMGA2, SNAIL	Breast cancer	56
miR-106a	TWIST	Hepatocellular carcinoma	58
miR-33a, miR-32	TWIST1	Lung cancer	61, 62

**Abbreviations:** CRKL, Crk-like protein; CtBP, C-terminal-binding protein; E-CAD, E-cadherin; EMT, epithelial-mesenchymal transition; EZH2, enhancer of zeste homolog 2; FOS, FBJ murine osteosarcoma viral oncogene homolog; Foxf2, forkhead box F2; HMGA2, high-mobility group AT-hook2; MET, pro-oncogene, receptor tyrosine kinase; MMP, matrix metalloproteinases; TCF4, transcription factor 4; TGFBR2, transforming growth factor beta receptor 2; LEF-1, Lymphoid enhancer-binding factor 1; VIM, vimentin.

units with invasive effects on PNA targeting miR-221 could significantly inhibit the invasive ability of breast cancer cell lines in vitro. In vivo experiments also verified that PNA-anti-miR-21 significantly inhibited tumor growth.

#### miR-214/miR-346 promoting EMT in lung cancer

In A549 and NCI-H1650 cell lines, ectopic expression of miR-214 promoted the development of EMT in a lung adenocarcinoma cell line.<sup>71</sup> The XPC (xeroderma pigmentosum complementation group C) expression is positively correlated with the outcome of NSCLC patients. However, it was found that miR-346 promoted the invasion and migration of NSCLC cells, which directly targeted XPC and then activated the ERK/SNAIL pathway, resulting in decreased expression of E-CAD.<sup>72</sup>

#### miR-21/miR-181a promoting EMT in liver cancer

In liver cancer, miR-21 inhibited PTEN and human sulfate-1 (sulfatase-1, hSulf-1) expression, resulting in activation of the AKT/ERK pathway and;<sup>73</sup> a similar effect was seen in cholangiocarcinoma,<sup>74</sup> and clear cell renal carcinoma.<sup>75</sup> In mice with experimental NSCLC, treatment for 35 days with oral silibinin significantly reversed high miR-21/low miR-200 levels in the neoplastic cells, thereby inhibiting EMT via decreasing downstream ZEB and N-CAD expression.<sup>76</sup> Applying bioinformatic analysis, Chang et al found 34 miRNAs expression upregulated in HCC, with miR-181a the most marked. To make a further study, 55 HCC tumor tissue samples and the adjacent normal tissues were selected. The qPCR assay verified increased miR-181a expression in tumor tissues, and the higher the



**Table 2** miRNAs known to promote EMT

miRNA promoting EMT	Target	Cancer types	References
miR-9	E-CAD	Esophageal squamous cell carcinoma, colon cancer	63, 64
	KLF17	Hepatocellular carcinoma	65
miR-373	HIF-1 $\alpha$ – TWIST	Breast cancer	67
miR-221	ATXN1	Breast cancer	68
miR-214	Sufu	Lung cancer	71
miR-346	XPC, SNAIL	Lung cancer	72
miR-21	PTEN, hSulf-1	Hepatocellular carcinoma, cholangiocarcinoma, clear cell renal cell carcinoma	73–75
miR-181a	PTEN	Hepatocellular carcinoma	77

**Abbreviations:** E-CAD, E-cadherin; EMT, epithelial–mesenchymal transition; Fbxo45, atypical ubiquitin E3 ligase complex; HIF-1 $\alpha$ , hypoxia-inducible factor; hSulf-1, human sulfate-1; KLF17, Krüppel-like factor 17; PTEN, phosphatase and tensin homolog deleted on chromosome ten; Sufu, suppressor-of-fused protein; XPC, xeroderma pigmentosum complementation group C.

stage of tumor, node, and metastasis, the higher level of miR-181a. MiR-181a knockdown reduced SNAIL, N-CAD, and MMP2/9, while increased E-CAD, accompanied by weaker invasiveness. It was proved that miR-181a promoted the occurrence of tumor EMT and promoted the invasion of HCC cells. In addition, they observed a decrease in P-AKT levels during the process. Therefore, it can be inferred that miR-181a regulates EMT process through phosphoinositide 3-kinase (PI3K)/AKT. Double luciferase assay revealed that PTEN was the target molecule of miR-181a.<sup>77</sup>

In conclusion, miRNAs promoting EMT significantly contribute to the invasiveness and metastatic tendency of neoplasms. They are potential targets of tumor therapy (Table 2).

## Effects of several common natural products on tumor EMT

### The general mechanisms of natural products in regulating EMT in tumor cell

There are some general mechanisms of natural drugs in regulating EMT in tumor cells. In the analysis of biopsies from the tumors of 178 gastric cancer patients, Yadav et al<sup>78</sup> found that TGF- $\beta$ 1 expression was negatively correlated with E-CAD ( $r=-0.452$ ,  $P<0.001$ ), and the Kaplan–Meier survival curves showed that prognosis of patients with low expression of E-CAD was poorer overall ( $P<0.001$ ). Notch, Wnt, Hedgehog (Hh), and TGF- $\beta$  are common signal pathways that can activate TWIST, SNAIL, SLUG, ZEB, and other EMT-related transcription factors, mediating the conversion of epithelial cells into mobile mesenchymal cells. This is related to tumor cell invasion and migration.<sup>79</sup> In addition, the Notch pathway interacts with the Hh pathway closely. Specifically, the Notch pathway can inhibit Hh through Hes-1-mediated Gli-1 transcription inhibition. Abnormal activation of Notch increased the protein level of Gli2, and the Hh signaling

pathway's role in promoting monocilia choroid plexus glial cells depends on the function of the Notch signaling pathway. Hh pathway inactivation also can inhibit the Notch pathway, and both mediate mesenchymal epithelial transition.<sup>80–84</sup> The nuclear factor kappa B (NF- $\kappa$ B) signaling pathway is also involved in EMT, by activating SNAIL, SLUG, ZEB1/2, and TWIST, and upregulating MMPs, fibronectin, VIM, and other mediators in mesenchymal tissue.<sup>85</sup> In addition, the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway is an important mechanism of tumor EMT, which can be regulated by botanicals (plant-derived medicinal agents). On the other hand, some botanical drugs directly regulate the EMT-related miRNAs to inhibit tumor invasion and migration. Recent researches have revealed Fra-1 (a member of activating protein-1 transcription factor complex) as a target of various natural products and it can be activated by STAT3. Both of them can be regulated by natural products such as curcumin, resveratrol, and luteolin in tumor EMT progression.<sup>86</sup>

### Curcumin

Curcumin inhibits hepatocyte growth factor-induced EMT in lung cancer cells (A549, PC9), restoring E-CAD and restraining N-CAD expression in a concentration-dependent manner, mainly through regulating the c-MET/PI3K/Akt/mTOR pathway. Thus, curcumin is thought to inhibit invasion and migration of lung cancer cells. Experiments in vivo also confirmed that injection of exogenous curcumin can repress neoplastic growth in tumor-bearing mice. Immunohistochemistry showed increased expression of E-CAD, while VIM, vascular endothelial growth factor (VEGF), and CD34 expressions were decreased, suggesting that curcumin may inhibit EMT and VEGF-mediated angiogenesis.<sup>87</sup>

Curcumin can inhibit migration of breast cancer stem cells in vitro through inhibiting the translocation of  $\beta$ -catenin

into the nucleus as well as the EMT-related transcription factor SLUG.<sup>88</sup> PAC (u4-hydroxy-3-methoxybenzylidene-N-methyl-4-piperidone) is a synthetic curcumin analog, with higher bioavailability. It can inhibit Erk1, AKT, and TWIST in breast cancer cells to control EMT *in vitro* and *in vivo*, thus inhibiting cell migration and invasion. Notably, its effect in estrogen receptor (ER)-negative cells (MDA-MB-231) is stronger than in ER-positive cells (MCF-7), which is of great significance for improving chemotherapeutic efficacy and prognosis of TNBC cells.<sup>89</sup> Bakiri et al<sup>90</sup> proposed that ectopic expression of Fra-1 induced mesenchymal phenotype transformation in completely polarized nontumorigenic mouse mammary epithelial Eph4 cells, with increased expression of TGF- $\beta$ 1, ZEB1, ZEB2, and slug. At the genetic level, Fra-1 could combine with the promoter of TGF- $\beta$ 1, ZEB2, and the conserved region in the first intron of ZEB1. In their experiments, they found that Fra-1-activating P3 fragment in ZEB2 promoter depended on three AP-1 binding sites, suggesting that Fra-1/AP-1 can regulate EMT-related transcription factor ZEB1 and ZEB2 expression through binding with their genome regulatory region. Previously, two Fra-1/AP-1 binding regions have been reported in human breast and CRC ZEB1 gene.<sup>91</sup> And curcumin could inhibit Fra-1/AP-1-mediated gene expression by inhibiting the binding of AP-1 to its cognate motif.<sup>92</sup> Liu et al<sup>93</sup> also found that curcumin could effectively reduce the low concentration of benzidine-induced EMT in human immortalized urothelial cell (SV-HUC-1 cell line) by inhibiting the ERK5/AP-1 pathway. Therefore, curcumin may inhibit the occurrence of EMT by repressing the expression of EMT-related transcription factors mediated by AP-1.

In cholangiocarcinoma, curcumin (EF24) inhibited NF- $\kappa$ B transcriptional activity in a concentration- and time-dependent manner. Moreover, it inhibited EMT, broke the cytoskeleton, and weakened the invasiveness and migration of tumor cells.<sup>94</sup> It was also found that diphenyl difluoroketone (EF24), a curcumin analog, inhibited EMT by upregulating miR-33b in cultured melanoma cells (in both the Lu1205 and A375 cell lines). The luciferase reporter gene showed that miR-33b could bind with HMG 3' UTR to repress its expression, and also regulated the formation of HMGA-2-dependent dynamic fibers and the activation of FAK, Src, and RhoA. All these effects contributed to inhibiting tumor metastasis.<sup>95</sup> A recent study indicated that EF24 inhibited phosphorylation of Src in HCC cells, then repressed tumor invasion and metastasis.<sup>96</sup>

Curcumin can also reverse EMT in the pancreatic cancer cell line PANC-1 and inhibit the invasion and migration of

tumor cells. This may be related to the inhibition of the Shh (sonic hedgehog)-GLI1 signaling pathway.<sup>97</sup> It was also found to block EMT progression in PANC-1 cell by inhibiting the PI3K/Akt/NF- $\kappa$ B pathway.<sup>98</sup> The antitumor mechanisms of curcumin in pancreatic cancer included upregulation of tumor suppressors, such as miR-26a, miR-101, miR-146a, miR-200b, miR-200c, and the LET-7 family, as well as downregulation of miR-21, which could contribute to EMT. It could induce cell apoptosis and inhibit cell proliferation, migration, and invasion.<sup>99–103</sup> The same effects were confirmed *in vivo*.<sup>97,100</sup> Isoflavone mixtures (G2535), synthetic 3,3'-bis (indolyl) methanes, or synthetic curcumin analogs all can inhibit miR-221-related proliferation and migration of pancreatic tumor cells.<sup>69</sup>

Curcumin may inhibit the Wnt signaling pathway and upregulate NKD2 expression to suppress EMT in colon cancer. In the detection of Wnt- and EMT-related molecular events, it was found that curcumin could inhibit TCF and  $\beta$ -catenin expression in the Wnt signaling pathway and promote Axin (the Wnt negative regulator) expression in a concentration-dependent manner. In addition, it has been shown that transfection of CXCR4 plasmid or NKD2 siRNA to SW620 cells reversed the inhibitory effects of curcumin on the Wnt signaling pathway and EMT, indicating that the mechanism of curcumin here was related to these two molecules as well.<sup>104</sup> Toden et al<sup>105</sup> found the same results and revealed that curcumin could downregulate the expression of ZEB1 and increase EMT inhibitory miR-200b, C, 141, 429, 101. More importantly, curcumin can induce epithelial expression in 5-fluoride-resistant cells to inhibit EMT, so as to ameliorate the drug resistance. This is of great value for improved therapeutic efficiency by curcumin. Montgomery et al<sup>106</sup> reported that curcumin and silymarin, two plant-derived agents used in colon cancer, can coordinate the antitumor effect. Treatment with curcumin alone can make the activity of cysteinyl aspartic enzyme 3/7 increase by a factor of 3, whereas in combination therapy with silymarin, the activity can be increased by a factor of 5.

## Genistein

Genistein is an isoflavone angiogenesis inhibitor and a phytoestrogen. In laryngeal carcinoma HEP-2 cells, genistein showed inhibitory effects on EMT, and significantly reduced cell invasion and migration *in vitro*.<sup>107</sup> The genistein treatment regimen produced the same findings in ASPC-1 pancreatic cancer cells, and mainly promoted miR-200b expression inhibited by the Notch-1 signaling pathway.<sup>108</sup> Nevertheless, it can downregulate miR-223 expression in GR pancreatic cancer

cells and this process can reverse EMT in GR cells, sharing the same effect with miR-223 inhibitor. Both of them can inhibit invasion and metastasis of GR cells and restore their sensitivity to gemcitabine.<sup>109</sup> Prostate cancer cells (ARCaP-E/ARCaP-M cells) are also sensitive to genistein.<sup>110</sup> It raised tumor suppressors such as miR-145 and miR-29a through methylation of the promoter, while miR-222, miR-221, and miR-151 (which stimulate tumor growth) were inhibited.<sup>111</sup> The inhibition of EMT was attributable to downregulation of the TGF- $\beta$  pathway in ovarian cancer.<sup>112</sup> GEN-27, a new synthetic isoflavone derived from genistein, can inhibit the nuclear translocation of NF- $\kappa$ B in THP-1 and HCT116 colon cancer cell lines. Wang et al<sup>113</sup> found that 100 nM Guqu trichostatin A combined with genistein significantly enhanced the antitumor effect of genistein; the potential mechanism may be related to AKT pathway inhibition. This kind of combined chemotherapy is an effective strategy for cancer treatment.<sup>107</sup>

## Resveratrol

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is isolated from red grape skins and can inhibit ZEB2 expression directly through epigenetic regulation in oral cancer.<sup>114</sup>

Lipopolysaccharide (LPS)-mediated EMT is closely related to inflammation-induced melanoma metastasis; Chen et al<sup>115</sup> found that resveratrol could inhibit LPS-induced tumor cell migration, accompanied by decreased EMT markers. The cellular mechanisms lay in weakened NF- $\kappa$ B nuclear localization and transcriptional activity.

Breast cancer cell EMT in MDA-MB-231 cells and MCF-7 cells is significantly inhibited by 3,6-dihydroxyflavone, by downregulating TWIST, SLUG, SNAIL, and N-CAD expression while decreasing E-CAD. As a result, tumor invasion, migration, and stem cell formation were inhibited. Further studies showed that Notch1 inhibition and miR-34a upregulation were involved.<sup>116</sup> Nevertheless, in tamoxifen-resistant MCF-7TR breast cancer cells, resveratrol reversed EMT by reducing endogenous TGF- $\beta$  and inhibiting SMAD phosphorylation.<sup>117</sup>

Oxidative stress and ROS induced by *Klebsiella pneumoniae* infection can lead to EMT in A549 cells and increase the expression of transcription factors, including TWIST, SNAIL, and ZEB ( $P < 0.01$ ). Nevertheless, the antioxidant effects of resveratrol inhibited these processes, and reduced tumor cell invasion and migration.<sup>118</sup> In osteosarcoma cells, resveratrol reduced hypoxia-induced EMT through downregulation of HIF-1 protein expression.<sup>119</sup>

Resveratrol in colon cancer LoVo cells mainly suppressed SNAIL expression mediated by the TGF- $\beta$ /SMAD signaling

pathway.<sup>120</sup> On the other hand, Buhrmann et al<sup>121</sup> found that resveratrol downregulated nuclear localization, phosphorylation, and acetylation of NF- $\kappa$ B in colon cancer HCT cells and showed epithelium induction in colon cancer HCT116 cells. These findings point to important mechanisms by which resveratrol can inhibit tumor invasion and migration.

The findings for prostatic carcinoma seem different to some degree, in that in these studies resveratrol directly inhibited the level of ERK-1/2, AKT protein, androgen, and insulin-like growth factor-1 receptors, thus increasing apoptosis and reducing proliferation and migration. In addition, resveratrol reduced the levels of several oncogene miRNAs including miR-17, miR-18, miR-20a, miR-20b, miR-926, miR-106b, miR-106a, and miR-7. In contrast, resveratrol increased tumor suppressors including miR-149, miR-150, miR-152, and miR-654-5p.<sup>122</sup> Treatment with 100  $\mu$ M resveratrol in cervical adenocarcinoma Hela cells could inhibit STAT3, Notch, and Wnt activation.<sup>123</sup> Kotha et al<sup>124</sup> first identified Src-STAT3 signaling as a target of resveratrol in malignant cells. As stated above, STAT3-Fra1 inhibition can exert a negative effect on EMT, which may be another mechanism of resveratrol's function.

## Catechin

Green tea catechins are water-soluble polyphenols, the most potent and abundant of which is EGCG (epigallocatechin-3-gallate). Catechins have been studied for possible use in cancer prevention and treatment. In oral squamous cell carcinomas (ALDH1<sup>+</sup> stem cells), decreased miR-204 expression correlated with the stem characteristics and lymph node metastasis. However, EGCG treatment could improve the expression of miR-204 and inhibit tumor-related EMT processes. It was also shown to restrict tumor growth in vivo.<sup>125</sup> Through its regulation on EMT and anti-angiogenesis effects, EGCG inhibited the invasion and migration of breast cancer cells.<sup>126</sup> In addition, EGCG competed for binding sites for ATP in the PI3K/mTOR active region and then blocked the PI3K/AKT/mTOR pathway.<sup>127</sup> Another study showed that EGCG mediated HMG-box transcription factor 1 (HBP1), which is an inhibitory factor, blocking the Wnt/ $\beta$ -catenin signaling pathway, and inhibiting the proliferation and migration of breast cancer cells.<sup>128</sup> TGF- $\beta$ /Smad pathway plays an important role in EMT process. Ko et al claimed that EGCG inhibited acetylation of Smad2 and Smad3 by p300/CBP during TGF- $\beta$ 1-induced EMT and reversed the upregulation of EMT-associated genes.<sup>129</sup> Activation of the MAPK family member Erk5 was involved in gastric cancer cell EMT mediated by tobacco. Lu et al fed 50 or 100 ng/mL

EGCG to mice that were exposed to cigarette smoke, and found EGCG treatment reversed EMT via inhibiting Erk5.<sup>130</sup> In HCT116 colon cancer cells, EGCG inhibited stem cell mass formation. Moreover, EGCG in 5FUR HCT116 and SW480 cells also inhibited the Notch pathway, which was closely related to tumor cell self-renewal, and downregulated CD44, CD133, Bmi1, and Notch1, which represented tumor stem characteristics. In addition, EGCG treatment increased miR-34a, miR-145, and miR-200c in colon cancer cells. These tumor-suppressing molecules may be one mechanism by which EGCG inhibits colon cancer EMT processes, invasion, migration, and chemoresistance.<sup>131</sup>

## Luteolin

Luteolin is a natural flavonoid compound with anticancer properties. Besides TGF- $\beta$ 1/Smad pathway, Chen et al<sup>132</sup> have verified that TGF- $\beta$  induced EMT via PI3K/Akt/NF- $\kappa$ B/SNAIL pathway in lung cancer A549 cells, and luteolin could inhibit this pathway and then repressed migration of A549 cells. Hypoxia can significantly reduce the expression of E-CAD but increase N-CAD, which indicates an EMT progression. However, luteolin treatment inhibited this hypoxia-inducible EMT through  $\beta$ -3 integrin/FAK signaling depression in malignant melanoma and NSCLC in vivo and in vitro.<sup>133,134</sup> Huang et al found that luteolin inhibited the expression of MMP2, MMP-7, and MMP-9 in PANC-1 and SW1990 cell lines in a concentration-dependent manner. IL-6 treatment increased the activity of STAT3, which could be blocked by luteolin.<sup>135</sup>

## Others

Cucurbitacin B reduced Wnt/beta-catenin levels, inhibited neoplastic cell migration, and angiogenesis, and reversed TGF- $\beta$ -induced EMT in NSCLC.<sup>136</sup> It also reduced mesenchymal molecular expression in a concentration-dependent manner.<sup>136</sup> When tested in human HCC cells PLC and Bel7402, apigenin inhibited EMT and increased cell adhesion by reducing NF- $\kappa$ B and SNAIL expression, and then inhibited the proliferation, invasion, and migration of tumor cells in a concentration-dependent manner.<sup>137</sup>

Mangostin-encapsulated PLGA nanoparticles also inhibited EMT in pancreatic cancer cell lines AsPC-1 and PANC-1, manifesting decreased expression of N-CAD and SLUG, as well as increased E-CAD expression. Simultaneously, cellular proliferation and migration were attenuated. The underlying mechanism was mainly attributed to inhibition of the Shh pathway.<sup>138</sup> It was shown that tripterine in ulcerative-colitis-associated colon cancer downregulated

SNAIL, VIM, and N-CAD, while raising E-CAD expression. At the same time, NF- $\kappa$ B activity was inhibited, which may be one of the mechanisms of EMT inhibition.<sup>139</sup>

Osthole is a bioactive coumarin extracted from medicinal plants. It can block EMT in androgen-independent prostate cancers and inhibits tumor invasion and metastasis. On the one hand, it reduces SNAIL production and targets the E-CAD promoter to inhibit EMT. On the other hand, it can significantly inhibit the production of miR-23a-3p, which is able to combine with E-CAD 3' UTR. Therefore, osthole inhibits invasion and metastasis by prostate cancer.<sup>60</sup>

From what we have stated above, we summarize the common natural products and their functions in detail as in Table 3.

## Conclusions and perspectives

An increasing number of studies into EMT have revealed its association with tumor invasion, metastasis, and drug resistance. Accordingly, some new strategies for tumor treatment aimed at EMT are also being developed. This may prove to be an important new direction for further research. Studies on miRNA have gradually revealed the key functions of noncoding RNAs on regulating human gene expression. Abnormal expression of these factors is intimately related to tumor development. EMT-inhibiting miRNAs often have the effect of inhibiting tumor invasion and metastasis, but are generally downregulated in tumors. Conversely, EMT-inducing miRNAs exhibit the opposite effect. Although a small proportion of miRNAs have been identified through biochemical or bioinformatics methodologies, the current state of knowledge about these agents is far from satisfactory. Further studies are needed to explain various biological functions of miRNAs in more detail. It would be good to develop a comprehensive knowledge of this system, which is of significance for tumor diagnosis and treatment, and of the appropriate biomarkers. In addition, natural products have long been recognized as valuable resources and starting compounds for the development of novel drugs, following the model of the many existing agents. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) and paclitaxel are excellent examples of medications derived from natural products. More and more research has revealed that tumor EMT can be regulated by natural products. Moreover, it has been shown that combined use of these natural products with chemotherapy significantly improves the therapeutic effects of the treatments. Isolating and purifying specific pharmacological components from the known assortment of traditional medicinal and natural products is still the



**Table 3** Natural products known to regulate EMT

Natural products	Mechanism	Cancer types	Cell lines	References
Curcumin	c-MET/PI3K/Akt/mTOR↓	Lung cancer	A549, PC9	87
	β-catenin, Slug↓	Breast cancer	MCF-7, T47D	88
	Erk1, AKT, TWIST↓	Breast cancer	MDA-MB-231 (ERα <sup>+</sup> or ERα <sup>-</sup> )	89
	Fra-1, ZEB↓		NIH3T3	92
	NF-kB↓	Cholangiocarcinoma	HuCC-T1, TFK-1, HuH28	94
	miR-33b↑	Melanoma	Lu1205, A375	95
	Shh-GLI1 pathway↓	Pancreatic cancer	PANC-1	97
	PI3K/AKT/NF-kB↓	Pancreatic cancer	PANC-1	98
	miR-26a, miR-101, miR-146a, miR-200b, miR-200c, LET-7 family↑	Pancreatic cancer	BxPC-3, MIAPaCa	99–103
	miR-21, miR-221↓		AsPC-1, MiaPaCa-2	
	Wnt pathway↓		PC-3	
	Wnt pathway↓	Colon cancer	SW620	104
	miR-200b, c, 141, 429, 101↑	Colon cancer	HCT116, SW480	105
		Laryngocarcinoma	HEP-2	107
Genistein	miR-200b↑	Pancreatic cancer	ASPC-1	108
	miR-223↓	Pancreatic cancer	AsPC-1, PANC-1	109
	Wnt↓	Prostate cancer	ARCaP-E, ARCaP-M	110
	miR-145, miR-29a↑	Prostate cancer	PC-3	111
	miR-222, miR-221, miR-151↓			
	TGF-β pathway↓	Ovarian cancer	BG-1	112
	NF-kB↓	Colon cancer	THP-1, HCT116	113
	ZEB2↓	Head and neck cancer		114
	NF-kB↓	Melanoma	K1735	115
	TGF-β/Smad↓	Breast cancer	MCF-7TR	116
Resveratrol	TWIST, SNAIL, ZEB↓	Lung cancer	A549	117
	HIF-1↓	Osteosarcoma	Saos-2	118
	miR-21↓	Hepatocellular carcinoma	SMMC7721, HepG2	119
	PTEN↑			
	TGF-β/Smad↓	Colon cancer	LoVo	120
	NF-kB↓	Colon cancer	HCT116	121
	ERK-1/2, AKT, Androgen, IGFsR↓	Prostate cancer		122
	STAT3, Notch, NF-kB↓	Cervical adenocarcinoma	Hela	123
	miR-204↑	Oral squamous cell carcinoma	OECMI	125
	PI3K/AKT/mTOR↓	Breast cancer	MDA-MB-231	127
	HBPI, Wnt/β-catenin↓	Breast cancer	MDA-MB-231	128
	Smad2,3↓	Lung cancer	A549	129
	Erk5↓	Gastric cancer		130
	Notch↓	Colon cancer	HCT116, SW480	131
EGCG	miR-34a, miR-145, miR-200c↑		HCT116, SW480	131
	PI3K/AKT/NF-kB↓	Lung cancer	A549	132
	β3 integrin↓	Malignant melanoma	BI6F10	133
	STAT3, MMP2/7/9	Pancreatic cancer	PANC-1, SW1990	135
	Wnt/β-catenin↓	Lung cancer	A549, H1299	136
	NF-kB, SNAIL↓	Hepatocellular carcinoma	PLC, Bel7402	137
	Shh pathway, Slug↓	Pancreatic cancer	AsPC-1, PANC-1	138
	NF-kB, SNAIL↓	Colon cancer	HCT116, HT-29	139
	SNAIL, E-CAD, miR-23a-3p↓	Prostate cancer	PC3, DU145	60
Luteolin	PI3K/AKT/NF-kB↓	Lung cancer	A549	132
	β3 integrin↓	Malignant melanoma	BI6F10	133
	STAT3, MMP2/7/9	Pancreatic cancer	PANC-1, SW1990	135
Cucurbitacin	Wnt/β-catenin↓	Lung cancer	A549, H1299	136
Apigenin	NF-kB, SNAIL↓	Hepatocellular carcinoma	PLC, Bel7402	137
Mang-NPs	Shh pathway, Slug↓	Pancreatic cancer	AsPC-1, PANC-1	138
Tripterine	NF-kB, SNAIL↓	Colon cancer	HCT116, HT-29	139
Osthole	SNAIL, E-CAD, miR-23a-3p↓	Prostate cancer	PC3, DU145	60

**Abbreviations:** E-CAD, E-cadherin; EGCG, epigallocatechin-3-gallate; EMT, epithelial-mesenchymal transition; HBPI, HMG-box transcription factor 1; NF-kB, nuclear factor kappa B; Shh, sonic hedgehog.

focus of relevant research. There is little doubt that this strategy is of great clinical relevance, suggesting that we can invest more effort in this work to achieve breakthroughs in tumor treatment.

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## Disclosure

The authors report no conflicts of interest in this work.

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