

## MINI REVIEW

## Research progress on the relationship between zinc deficiency, related microRNAs, and esophageal carcinoma

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### Introduction

Esophageal cancer (EC) is a common gastrointestinal malignancy that includes adenocarcinoma (EAC) and squamous cell carcinoma (ESCC), with ESCC being the predominant histological subtype. Because early clinical symptoms are lacking, a diagnosis of EC often occurs at an advanced stage and is subsequently accompanied by a poor prognosis. Although recent developments have been made in various techniques, such as surgery, radiotherapy, and chemotherapy, the survival rate of EC patients remains unsatisfactory, with an overall five-year survival rate of approximately 20.9%. Thus, there is an urgent need to clarify the underlying mechanism of the pathogenesis of EC and to search for novel targeted methods of treatment. Zinc (Zn) is an essential trace element required for the activity of ~2000 transcription factors and >300 enzymes. Dietary Zn is necessary for normal physiological development, including growth, reproduction, pregnancy development, and immune system regulation.<sup>1,2</sup> A number of studies have found that Zn deficiency plays an important role in the occurrence and development of EC. We provide a review of recent advances in determining the molecular relationship between Zn deficiency and EC to

### Abstract

Esophageal cancer (EC) is a common malignant tumor of the gastrointestinal tract with a high incidence in China. Zinc (Zn) deficiency is a key risk factor for the occurrence and development of EC and affects progression by regulating microRNA (miRNA, miR) expression. In addition, the dysregulation of miRNAs is accompanied by the dysregulation of their target genes in EC. In this paper, we review the potential molecular mechanisms between Zn deficiency and EC with the aim of providing new strategies and methods for early diagnosis, targeted therapy, and prognostic evaluation.

provide new ideas and methods for prevention and treatment.

### Zn deficiency induces EC occurrence

GLOBOCAN 2012 data revealed that EC is the eighth most common cancer worldwide, with an estimated 456 000 new cases and 400 000 deaths in 2012, accounting for 3.2% and 4.9% of all cancer incidence and mortality, respectively.<sup>3</sup> In China, EC is the fifth most common cancer, with an estimated 223 000 new cases and 197 000 deaths, accounting for 49% and 49.3% of the total new cases and deaths worldwide, respectively.<sup>3,4</sup> Moreover, Hebei provincial Cancer Registry Center data showed that EC was the fourth most common cancer in 2012, with an estimated 15 100 new cases and 10 700 deaths.<sup>5</sup> Cixian and Shexian are high-risk areas for EC in Hebei province and the incidence in Cixian is the highest in both China and the world. EC incidence has clear geographical distribution characteristics.

Etiology studies have shown that the occurrence of EC is a result of a variety of factors, including genetic factors, smoking, excessive drinking, local esophageal injury, exposure to nitrosamine carcinogens, a lack of vitamins and trace

elements, and mold and viral infections.<sup>6</sup> Zn deficiency is a common phenomenon in the high incidence of EC and plays a key role in initiation and progression. Zou *et al.* analyzed the nutrient intake of residents of Linxian, a high-risk area for EC in China, and reported that in 1996, serum Zn levels were only 72% and 62% of the recommended dietary allowance (RDA) in the spring and autumn, respectively.<sup>7</sup> Lu *et al.* performed a case-control study including 218 EC patients and 415 healthy controls using a standard dietary and health questionnaire. Their results suggested that Zn deficiency contributed to EC risk and an increased dietary intake of Zn may decrease such risk in a Chinese population.<sup>8</sup> Torin *et al.* assessed the association between the EC incidence rate and dietary nutrient supplies in 32 African countries and found that the mean supply of Zn was lower in high-risk than in low-risk countries.<sup>9</sup> In 2015, an analysis of the Golestan cohort study, which examined 201 EC patients among 47 405 patients, suggested a significant negative correlation between the level of Zn and the incidence of EC (hazard ratio [HR] 0.71, 95% confidence interval [CI] 0.49–1.03).<sup>10</sup> Maryam *et al.* randomly selected 30 nail samples of EC patients from the participants of the Golestan cohort study and demonstrated that the Zn level in nail samples could be used as a biomarker of EC.<sup>11</sup> A comparative study performed by Ray *et al.* in both high and low areas of EC incidence showed that the levels of Zn in hair and food grain were significantly reduced in high-incidence compared to low-incidence areas.<sup>12</sup> In a systematic review of Zn biomarkers and EC risk including 10 case-control studies, one prospective cohort study and eight clinical trials showed an inverse association between Zn concentration and EC incidence and a higher Zn status was associated with a reduced risk of EC.<sup>13</sup> In 2017, He *et al.* reported that the serum Zn levels of residents from high-incidence and low-incidence areas were  $87.20 \pm 5.67 \mu\text{g}/100 \text{ mL}$  and  $111.10 \pm 5.11 \mu\text{g}/100 \text{ mL}$ , respectively. The levels of serum Zn in residents from high-incidence areas were significantly lower than those from low-incidence areas, and the levels of serum Zn in EC patients were lower than healthy volunteers, regardless of whether they were from a high-incidence or low-incidence area.<sup>14</sup> In addition, Hamidreza *et al.* collected 240 fasting serum samples from eastern and western regions of Golestan province that were considered high and low-risk regions for EC and found that the mean serum level of Zn in the high-risk region was significantly lower than that in the low-risk region.<sup>15</sup>

### Zn deficiency affects the development of EC by regulating miRNA expression

Micro-RNAs (miRNAs, miRs) are non-coding RNAs approximately 18–22 nucleotides in length that post-transcriptionally regulate gene expression by base-pairing

to partially complementary sequences in the 3'-untranslated region (UTR) of their target messenger RNA (mRNA). Previous studies have shown that miRNA dysregulation is involved in the development of EC and that miRNAs act as both oncogenes and tumor suppressor genes.<sup>16–18</sup>

He *et al.* divided human EC cell lines KYSE170 and ECA109 into two groups: one cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and the other in RPMI 1640 medium containing 10% FBS plus  $50 \mu\text{M}/\text{L}$   $\text{ZnSO}_4$ . The miRNA levels in the two cell lines were then measured to analyze the influence of the Zn level on the miRNAs. The miRNA expression levels in EC cells cultured without Zn were used as standards. The relative levels of miR-21, miR-31, and miR-93 were significantly decreased, while the expression of miR-375 was significantly increased in EC cells cultured with Zn, with no difference in cell status. These results indicated an obvious correlation between Zn level and miRNA expression in EC.<sup>14</sup>

In animal experiments, Alder *et al.* found that miR-31, miR-21, miR-142-3p, and miR-223 were significantly upregulated, while miR-375 and miR-203 were significantly downregulated in rats with Zn-deficient EC compared to Zn-sufficient EC. MiR-21 overexpression was accompanied by downregulation of its tumor suppressors programmed cell death 4 (PDCD4) and tropomyosin  $\alpha$  1 chain (TPM1), whereas miR-31 overexpression was accompanied by downregulation of its tumor suppressor protein, phosphatase 2 regulatory subunit Balpha (PPP2R2A) in rats with Zn-deficient EC.<sup>19</sup> Fong *et al.* found that EC incidence is highest in severely Zn deficient rats and that severe Zn deficiency alone induced a highly proliferative/inflammatory esophagus accompanied with oncogenic 5-miRNA signatures (miR-31, miR-223, miR-21, miR-146b and miR-146a; up 3.7- to 4.9-fold) (Table 1).<sup>20,21</sup>

Wen *et al.* investigated the expression of miR-21 in 76 pairs of human EC tissues and adjacent para-cancerous tissues and the EC cell line, TE-13. MiR-21 expression was significantly increased in EC tissues and EC TE-13 cells. In addition, miR-21 expression in the phase T3/T4 samples was remarkably higher than in phase T1/T2 samples, suggesting that higher miR-21 expression predicts deeper tumor invasion. MiR-21 expression in EC tissues in patients with lymph node metastasis was significantly higher than in patients without lymph node metastasis. The results also showed that high miR-21 expression in TE-13 cells increased cell proliferation and invasion and inhibited apoptosis.<sup>22</sup>

Komatsu *et al.* conducted in vivo testing to ascertain whether oncogenic miR-21 promoted chemoresistance in EC patients and acted as a biomarker for predicting chemoresistance in plasma from patients with EC. All EC

**Table 1** Levels of dietary Zn deficiency in the development of esophageal cancer

Zn deficiency	Tumor incidence (%)	Cell proliferation (%)	Inflammatory factors	miRNAs	Expression of multiple
Severe Zn deficiency (3 mg Zn/kg)	100.0	49 ± 2.9	Cxcl5, Cxcl2, Cxcl3, Csf3, Il17f, Cxcl1, Ceacam1, Ccl2, Ccr1, Ccr2, Cxcr4, Mmp9, Tlr4, Tlr2, Cox-2, Il1b, Nfkb1, Hif1a, S100A8, S100A9	miR-31,-223,-21, -146b, -146a	Up 3.7 to 4.9
Moderate Zn deficiency (6 mg Zn/kg)	72.0	39 ± 2.7	S100A8, S100A9, Cxcl5, Ptgs2, Cxcl2, Ilb1	miR-146a,-31, -146b,-27a	Up 1.4 to 1.9
Mild Zn deficiency (12 mg Zn/kg)	72.0	31 ± 4	S100A8, S100A9, Cxcl5, Ptgs2, Cxcl2, Ilb1	miR-146a,-31, -223	Up 1.4 to 1.8

Adapted from Fong LY *et al.*<sup>20</sup> and Taccioli C *et al.*<sup>21</sup>

patients were treated with a preoperative chemotherapy regimen of cisplatin plus 5-fluorouracil (5-FU). The plasma miR-21 expression level was significantly higher in EC patients administered the preoperative chemotherapy regimen who had a low histopathological response compared to patients who had a high histopathological response, suggesting that high plasma miR-21 expression predicts a low histopathological response to chemotherapy. In vitro, the EC cell line KYSE170 was transfected with miR-21 mimics to increase miR-21 expression, and miR-21 upregulation was associated with decreased chemosensitivity to 5-FU or cisplatin treatment. In summary, miR-21 overexpression contributed to chemoresistance and circulating miR-21 in the plasma of EC patients could serve as a useful biomarker for predicting chemoresistance.<sup>23</sup>

Wen *et al.* conducted a meta-analysis of five studies including 504 subjects to explore the association between miR-21 expression and prognosis in patients with EC. Their results suggested that high miR-21 expression was associated with a poorer prognosis for patients with EC (pooled HR 1.87, 95% CI 1.37–2.55;  $P < 0.001$ ).<sup>24</sup> Zhang *et al.* measured miR-31 expression in 45 pairs of EC tissues and corresponding normal tissues and 523 serum samples and found that miR-31 was upregulated in EC tissues compared to corresponding normal tissues and high miR-31 expression was positively correlated with tumor invasion depth, tumor node metastasis (TNM) stage, and metastasis. Serum miR-31 expression levels in EC patients were also significantly higher than in normal controls. Follow-up data showed that patients with high miR-31 expression had a poorer prognosis for relapse-free and tumor-specific survival. Univariate and multivariable Cox regression analyses both revealed that serum miR-31 expression was an independent prognostic biomarker for EC. The experiment also showed that miR-31 promoted colony formation, migration, and invasion of EC cell lines EC9706, KYSE510, and KYSE150 in vitro.<sup>25</sup>

Matteo *et al.* measured miR-223 expression in 280 gastroesophageal biopsy samples representative of the whole

spectrum of phenotypic changes involved in carcinogenetic cascades. MiR-223 expression was significantly increased along with the severity of the considered lesions in both gastric and esophageal models. Moreover, miR-223 overexpression was clearly relevant to intestinal metaplasia scores in atrophic gastritis and Barrett esophagus samples. MiR-223 plasma levels were significantly higher in patients with cancer than in controls. Taken together, these findings suggest that miR-223 overexpression plays a pivotal pathogenic role starting from the earliest phenotypic changes of gastroesophageal carcinogenesis and may serve as a new biomarker in the secondary prevention strategy for both gastric cancer and Barrett adenocarcinoma.<sup>26</sup>

Hu *et al.* investigated the expression of miR-375 and its underlying molecular mechanism in EC. Ten pairs of EC tissues and adjacent para-cancerous tissues, as well as the human EC cell line EC109, and a normal esophageal epithelial cell line, Het 1A, were examined. The miR-375 level was significantly downregulated in primary EC tissues compared to adjacent para-cancerous tissues, and its expression in EC109 cells was significantly decreased compared to Het 1A cells. After transfection with a miR-375 mimic, miR-375 overexpression inhibited EC109 cell proliferation and invasion and induced cell cycle arrest compared to the negative control group.<sup>27</sup>

Ansari *et al.* investigated the expression of miR-93 in 30 pairs of tumor tissues and adjacent para-cancerous tissues and evaluated their diagnostic and therapeutic potential in EC. MiR-93 expression was significantly increased in primary tumor tissues compared with adjacent para-cancerous tissues, suggesting that miR-93 can be used as a biomarker in EC.<sup>28</sup>

Lv *et al.* examined the expression of miR-21 and miR-375 in 126 patients with EC and 80 healthy volunteers. The miR-21 level was significantly higher in cancer tissues than in adjacent para-cancerous tissues, while the miR-375 level was lower. Serum miR-21 expression was significantly elevated in EC patients compared to healthy controls, while the serum miR-375 level was significantly lower. The miR-

21 expression level was significantly associated with tumor infiltration, vascular invasion, and lymph node metastasis, and the miR-31 expression level was remarkably correlated with TNM stage, vascular invasion, and lymph node metastasis. Moreover, miR-21 expression was closely linked to radiotherapy sensitivity and EC recurrence. Patients with high miR-21 expression had lower radiotherapy sensitivity than those with low miR-21 expression, accompanied with a higher probability of recurrence. The results also revealed that miR-21 and miR-375 expression levels and the miR-21/miR-375 ratio could act as novel serum markers for early diagnosis and predict prognosis in EC, as well as novel drug targets and determining appropriate candidates.<sup>29</sup>

Wu *et al.* compared miR-223 and miR-375 expression levels in 20 pairs of EC and matched para-cancerous tissues, as well as serum samples from 94 healthy volunteers and 194 patients with EC. MiR-223 expression was significantly increased, while miR-375 expression was decreased in EC tissues compared to para-cancerous tissues. Serum miR-223 expression was significantly higher and serum miR-375 expression was significantly lower in EC patients than in healthy controls, respectively. Survival analysis indicated that high serum miR-223 expression and low miR-375 expression were associated with poor survival and could act as independent prognostic biomarkers for EC patients.<sup>30</sup> In addition, a meta-analysis of 22 studies, including a total of 1946 EC patients from Canada, China, Germany, Japan, and the United States indicated that high miR-21 expression had a negative impact on overall survival (OS; HR 1.52, 95% CI 1.17–1.98;  $P = 0.001$ ), whereas low miR-375 expression had a negative impact on OS (HR 0.53, 95% CI 0.39–0.73;  $P < 0.001$ ). These results indicate that miR-21 upregulation and miR-375 downregulation can predict an unfavorable prognosis in EC patients.<sup>31</sup>

### Upregulation of oncogenic miR-223, miR-31, and miR-21 is accompanied by downregulation of their tumor suppressor targets in EC

Fong *et al.* demonstrated that Zn deficiency upregulated miR-21, miR-31, and miR-223 expression, which was accompanied by downregulation of their tumor suppressor targets, *PDCD4*, *serine/threonine kinase 40 (STK40)*, and *F-box and WD repeat domain-containing 7 (FBXW7)* in a Zn deficient rat EC model.<sup>20</sup> The *FBXW7* gene locus, which is located at chromosome 4q32, is frequently decreased in a variety of human tumors. *FBXW7* coordinates the ubiquitin-dependent proteolysis of several critical cellular regulators, thereby controlling essential biological processes, including cell cycle, differentiation, and apoptosis. *FBXW7* is a cell cycle protein that regulates the stability of several oncoproteins, including c-Myc, cyclin E, c-Jun,

SREBP, Notch, and c-Myb, thereby acting as a vital tumor suppressor gene.<sup>32</sup> Multiple studies have reported that *FBXW7* expression is regulated by several miRNAs, such as miR-223, miR-25, miR-182, and miR-367.<sup>32,33</sup>

Kurashige *et al.* examined miR-223 and *FBXW7* expression levels in 109 pairs of primary EC tissue samples and corresponding normal esophageal epithelium samples obtained from patients who underwent esophageal resection without preoperative treatment. The miR-223 expression level was significantly higher in primary cancer tissues than in corresponding normal tissues. High miR-223 expression was associated with gender, tumor size, and tumor invasion depth in EC patients and predicted a significantly poorer prognosis. Kurashige *et al.* also examined *FBXW7* protein expression using immunohistochemical analysis of EC patient samples and found that *FBXW7* expression was downregulated in most EC tissues. There was a significantly inverse association between miR-223 expression and *FBXW7* protein levels in clinical EC patient samples: high miR-223 expression predicted low *FBXW7* expression. In vitro, the expression levels of miR-223 and *FBXW7* were tested in eight human EC cell lines (TE1, TE4, TE6, TE8, TE9, TE10, TE14, and TE15). The results revealed a significant inverse correlation between miR-223 expression and *FBXW7* mRNA level, consistent with the results of vivo experiments. *FBXW7* mRNA expression was also significantly decreased, while c-Myc and c-Jun protein levels were enhanced in TE6 and TE15 cells following transfection with pre-miR-223 compared to those transfected with the negative control. Conversely, the *FBXW7* mRNA expression level was significantly increased, while the c-Myc and c-Jun protein levels were deregulated in TE4 and TE14 cells transfected with anti-miR-223.<sup>34</sup> Taken together, miR-223 downregulates the expression of its tumor suppressor target *FBXW7* in EC.

Streppel *et al.* examined miR-223 expression in 95 pairs of EC tissues and matched para-cancerous tissues and found that miR-223 was upregulated in the EC tissues. They also detected the miR-223 expression level in EC cell lines OE33 and JHesoAD1 and the normal esophageal cell line HEEpiC. Their results suggested that miR-223 expression is upregulated in EC cell lines OE33 and JHesoAD1 relative to HEEpiC cells and that miR-223-transfected cells exhibited a statistically significant increase in migratory and invasive potential. Moreover, the study found that *poly (ADP-ribose) polymerase 1 (PARP1)* and *SMARCD1* were the target genes of miR-223 in EC, and miR-223 directly binds to their 3'-UTRs.<sup>35</sup> In summary, miR-223 is upregulated and is accompanied by the downregulation of its target tumor suppressor genes *FBXW7*, *PARP1*, and *SMARCD1* in EC. *STK40* is a negative regulator of nuclear factor kappa B (NF- $\kappa$ B)-mediated transcription and acts as a tumor suppressor directly targeted by miR-31.<sup>36–38</sup>

Taccioli *et al.* simulated the features of human EC using a rat model and examined the mechanism whereby Zn regulates miR-31 expression to promote EC progression. Zn deficiency induced the overexpression of miR-31 in a rat EC model. The researchers found that *in vivo*, anti-miR-31 reduced miR-31 expression. Moreover, miR-31 inhibition prominently suppressed the development of a hyperplastic esophageal phenotype in Zn-deficient rats by reducing cell proliferation and inducing cell apoptosis. MiR-31 directly interacts with the 3'-UTR of STK40, a negative regulator of NF- $\kappa$ B-mediated transcription. MiR-31 overexpression is significantly associated with STK40 protein downregulation and regulation of the NF- $\kappa$ B p65-RAGE-S100A9 inflammatory pathway. Furthermore, the same relationship between miR-31 overexpression and STK40/NF- $\kappa$ B expression was also documented in human EC cell lines KYSE450, KYSE410, KYSE520, and KYSE510 *in vitro*. Both the *vivo* and *vitro* experiments showed important relationships between miR-31, STK40, and NF- $\kappa$ B in EC tissue and human EC cells for the first time. In general, miR-31 overexpression is accompanied by the downregulation of its tumor suppressor *STK40* in a Zn-deficient esophagus.<sup>39</sup> Zhang *et al.* found that miR-31 was upregulated in EC tissues and serum samples compared to adjacent para-cancerous tissues and normal controls, respectively. High miR-31 expression promotes colony formation and the migration and invasion of EC cell lines EC9706, KYSE150, and KYSE510. High serum miR-31 levels also predict a poorer prognosis. Luciferase activity and Western blot analysis confirmed that the tumor suppressor genes *epithelial membrane protein 1 (EMP1)*, *kinase suppressor of ras 2 (KSR2)* and *regulator of G-protein signalling 4 (RGS4)* are targeted by miR-31.<sup>25</sup> Collectively, these results indicated that miR-31 is upregulated and is accompanied by downregulation of its target tumor suppressor genes *STK40*, *EMP1*, *KSR2*, and *RGS4* in EC. MiR-21 is one of the most consistently overexpressed onco-miRs in EC.<sup>40</sup>

Liao *et al.* detected the expression level of plasma miR-21 in 70 EC patients and 70 healthy volunteers and found that plasma miR-21 expression was higher in EC patients than in healthy controls. Exosome-shuttling miR-21 overexpression promoted the migration and invasion of EC cells by targeting *PDCD4* and activating its downstream c-Jun N-terminal kinase (JNK) signaling pathway. Exosome-shuttling miR-21 overexpression also inhibited expression of the tumor suppressor gene *PDCD4* at the translational level and affected EC progression.<sup>41</sup> Li *et al.* detected miR-21 and phosphatase and tensin homolog (PTEN) expression levels in 76 pairs of invasive EC tissues at stage I–IV and their corresponding para-cancerous histological normal tissues and assessed the effect of miR-21 on PTEN expression in EC cell lines. They also compared the miR-

21 expression levels in EC cell lines EC9706, EC-1, KYSE170, KYSE410, and KYSE180 to the human esophageal epithelial cell line HEEC. MiR-21 expression was significantly higher in EC lines and primary tumor tissues than the esophageal epithelial cell line (HEEC) and corresponding para-cancerous normal tissues. MiR-21 overexpression is also related to vascular invasion and advanced clinical TNM stage in EC patients. Kaplan–Meier analysis showed that high miR-21 expression predicted a significantly lower disease-free survival in EC tissues than low miR-21 expression, suggesting that miR-21 expression is an unfavorable predictor of the survival in EC patients. Li *et al.* also found that PTEN protein expression was significantly downregulated in tumor tissues. A statistically significant inverse relationship was observed between miR-21 expression and PTEN protein, with high miR-21 expression correlating with low PTEN protein expression. The downregulation of PTEN protein was significantly associated with vascular invasion, tumor status, lymph node metastasis, and clinical stage. The results also suggested that miR-21 knockdown significantly increased PTEN protein expression, which was validated in EC cell line EC9706 transfected with a miR-21 inhibitor. Consequently, PTEN overexpression reduced cell proliferation, invasion, and migration of EC. *PTEN* is a direct target of miR-21 and miR-21 suppressed PTEN expression by directly binding to the 3'-UTR of its mRNA.<sup>42</sup>

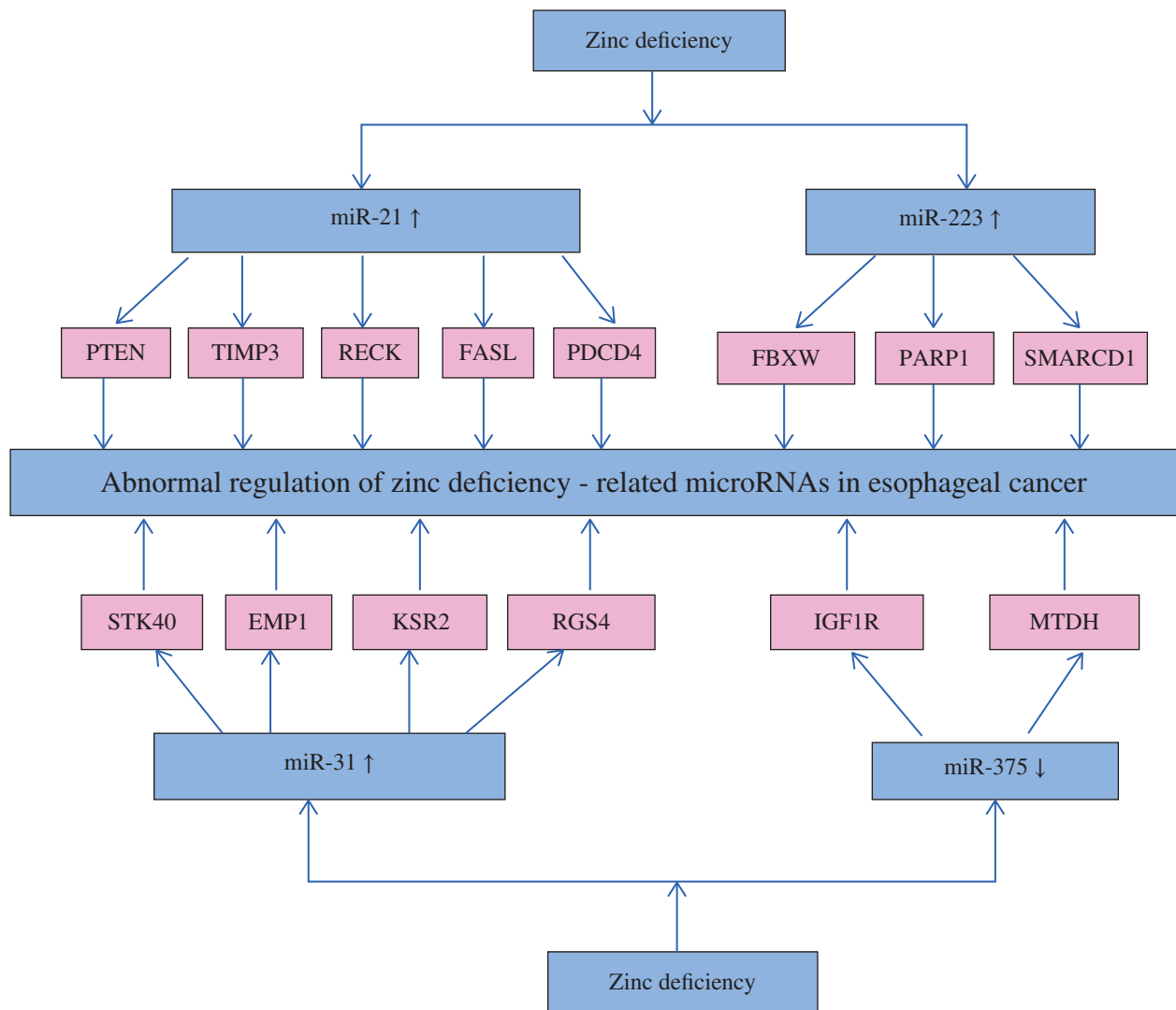
Wu *et al.* detected the expression of miR-21, PTEN, phosphatidylinositol 3-kinase (PI3K), and serine/threonine kinase (AKT) in 89 EC samples and 58 adjacent normal tissues. MiR-21 expression was significantly higher in human EC tissues than in adjacent normal tissues. PTEN protein expression was significantly downregulated, while PI3K and AKT protein expression were significantly upregulated in EC tissues relative to adjacent normal tissues, especially in EC tissues with lymph node metastasis and poor differentiation. Downregulation of miR-21 efficiently inhibited proliferation, migration, and invasion, and increased cell apoptosis in the EC cell line TE11 transfected with a miR-21 inhibitor. Downregulation of miR-21 significantly upregulated PTEN expression but downregulated PI3K and AKT protein expression in the EC cell line TE11 after transfection with a miR-21 inhibitor. *PTEN* is one of the negative target genes of miR-21 that can bind to the 3'-UTR of PTEN mRNA.<sup>43</sup> Wang *et al.* examined miR-21 expression in 16 pairs of primary EC tissues and adjacent para-cancerous tissues and discovered that miR-21 was significantly upregulated in EC tissues and high miR-21 expression was associated with lymph node metastasis in EC patients. Silencing of miR-21 expression in EC cell lines EC9706 and EC-1 significantly inhibited cell proliferation and invasion and promoted apoptosis *in vitro*. Importantly, *fas ligand (FASL)*, *tissue inhibitor of metalloproteinase 3*

(TIMP3), and *reversion-inducing-cysteine-rich protein with kazal motifs (RECK)* expression was increased after miR-21 downregulation. Moreover, miR-21 modulated *FASL*, *TIMP3*, and *RECK* expression by directly binding to the 3'-UTR of these genes.<sup>44</sup> In summary, miR-21 is significantly upregulated and is accompanied by downregulation of its target tumor suppressor genes *PDCD4*, *PTEN*, *FASL*, *TIMP3*, and *RECK* in EC.

In addition, Hu *et al.* detected miR-375 expression in 10 pairs of EC tissues and adjacent para-cancerous tissues, as well as the esophageal cell line EC109 and a normal esophageal epithelial cell line, Het 1A. MiR-375 was downregulated in EC tissues and EC109 cells compared to adjacent para-cancerous tissues and Het 1A cells. MiR-375 overexpression inhibited cell proliferation and invasion and induced cell cycle arrest in EC109 cells transfected

with a miR-375 mimic. MiR-375 overexpression also downregulated the expression of metadherin (MTDH) by binding to the 3'-UTR of MTDH mRNA in EC109 cells. The *MTDH* downstream protein vascular endothelial growth factor (VEGF-C) and cell cycle regulator cyclin D1 were decreased following treatment with a miR-375 mimic. In contrast, the miR-375 mimic significantly elevated the expression of the epithelial marker E-cadherin, which may inhibit tumor cell epithelial mesenchymal transition and invasion. These results suggest that *MTDH* is a direct target gene of miR-375.<sup>26</sup>

Kong *et al.* investigated miR-375 expression in 105 pairs of primary EC tissues and corresponding para-cancerous tissues, as well as in eight EC cell lines (KYSE30, KYSE140, KYSE180, KYSE410, KYSE510, KYSE520, EC109, and HKESC1). MiR-375 was significantly downregulated in EC



**Figure 1** Dysregulation and mechanism of microRNAs in carcinogenesis and the development of esophageal cancer.

tissues and cell lines and was significantly associated with advanced stage, distant metastasis, and poor OS and disease-free survival. MiR-375 suppressed tumor cell proliferation, invasion, and metastasis and downregulation of miR-31 was mostly caused by promoter hypermethylation. Moreover, there was a negative correlation between miR-375 and *IGF1R* in EC cell lines; miR-375 downregulated *insulin-like growth factor 1 receptor (IGF1R)* expression at both the mRNA and protein levels by binding to the 3'-UTR of *IGF1R* in vitro. *IGF1R* expression was significantly higher in primary EC tissues compared to the corresponding para-cancerous tissues. At the same time, a significant negative correlation was observed between miR-375 and *IGF1R* in EC and validated the result in vitro. *IGF1R* could serve as a direct downstream target of miR-375.<sup>45</sup> These results indicate that miR-375 is downregulated and is accompanied by upregulation of its target genes *MTDH* and *IGF1R* in EC.

In summary, Zn deficiency upregulates oncogenic miR-21, miR-31, and miR-223 and downregulates the tumor suppressor gene miR-375, all of which are accompanied by the dysregulation of their target genes in EC (Fig 1).

Furthermore, Zn deficiency also affected EC development by regulating the expression of certain coding genes, such as *NF-κB p65*, *cyclooxygenase-2 (COX-2)*, and *leukotriene A4 hydrolase (LTA4H)*.<sup>46</sup> Zn deficiency upregulated the expression of the Zn-sensitive gene *metallothionein-1 (MT-1)*, *cytokeratin 14 (KRT14)*, *carbonic anhydrase II (CAII)*, and *cyclin B*, and downregulated the expression of *calponin 1 (CNN1)*. Importantly, within 48 hours of Zn replenishment, the dysregulation of these genes returned to near Zn-sufficient levels.<sup>47</sup> Fong *et al.* confirmed that Zn gluconate supplementation for eight weeks led to a shift to a less proliferative/aggressive cancer phenotype by reducing cell proliferation, stimulating apoptosis, and decreasing expression of the key tumor markers *cyclin D1*, *p53*, and *COX-2* in a rat esophageal cancer model.<sup>48</sup> Taccioli *et al.* found that short-term Zn deficiency (six weeks) in rats induced overexpression of proinflammatory genes *S100A8* and *S100A9* in the esophageal mucosa accompanied with esophageal epithelial hyperplasia.<sup>49</sup> Long-term Zn deficiency (21 weeks) significantly increased the incidence of EC by inducing the overexpression of more inflammatory factors, while Zn supplementation reversed this process.<sup>21</sup>

Esophageal cancer is a common gastrointestinal malignancy with a poor prognosis. EC development is largely influenced by lifestyle factors, such as tobacco smoking, alcohol, and a lack of vitamins and trace elements. Accumulating evidence suggests that Zn deficiency plays an important role in the initiation and development of EC. Zn deficiency induces miRNA dysregulation, thus promoting the initiation and development of EC. Recent studies have

made significant progress determining the relationships between Zn deficiency, miRNA dysregulation, and EC, but the exact mechanisms remain unclear. Therefore, there is an urgent need to clarify the molecular mechanisms of these relationships, which will be beneficial for the targeted treatment and prognosis of EC. In this review, we summarized the mechanism of Zn deficiency, miRNAs and their related target genes in EC, in order to elucidate the etiology to prevent the development of EC.

## Disclosure

No authors report any conflict of interest.

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