REVIEW

Roles of microRNAs in tumorigenesis and metastasis of esophageal squamous cell carcinoma

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Abstract

Esophageal squamous cell carcinoma (ESCC) is the major subtype of esophageal cancer that is prevalent in Eastern Asia. Despite recent advances in therapy, the outcome of ESCC patients is still dismal. MicroRNAs (miRNAs) are non-coding RNAs which can negatively modulate gene expression at the post-transcriptional level. The involvement and roles of miRNAs have become one of the hot topics of cancer research in recent years. In ESCC, genetic variations within miRNA coding genes were found to have distinct epidemiological significance in different populations. Dysregulated expression of several miRNAs was reported to be associated with therapeutic response. Functionally, miRNAs can act either in an oncogenic or a tumor-suppressive manner during tumorigenesis of ESCC by interrupting signaling pathways associated with cell proliferation, metabolism, cancer stemness, and resistance to chemo- or radiotherapy. Moreover, miRNAs modulate metastasis of ESCC by targeting genes that regulate cytoskeleton dynamics, extracellular matrix remodeling, epithelial-mesenchymal transition, and tumor microenvironment. Most importantly, mounting evidence suggests that inhibiting oncogenic miRNAs or restoring the loss of tumor-suppressive miRNAs has therapeutic potential in the treatment of ESCC. Here, we review and discuss recent studies on the significance, biological functions, and therapeutic potential of miRNAs in tumorigenesis and metastasis of ESCC.

Key Words: MicroRNAs; Dysregulation; Tumorigenesis; Metastasis; Therapeutic potential; Esophageal squamous cell carcinoma

Core Tip: Esophageal squamous cell carcinoma (ESCC) is a deadly disease worldwide. Its poor prognosis is mainly due to the rapid tumor progression and high rate of

invasion and metastasis. It is of great importance to understand the mechanisms underlying ESCC tumorigenesis and metastasis. Increasing studies confirmed the involvement of microRNAs (miRNAs) in cancer progression. Dysregulated miRNAs can serve as possible biomarkers for ESCC diagnosis or prognosis evaluation. Moreover, miRNAs function as small post-transcriptional regulators with notable therapeutic value. This review summarizes recent studies on the significance, biological functions, and clinical potential of miRNAs in tumorigenesis and metastasis of ESCC.

INTRODUCTION

Esophageal cancer is one of the most aggressive cancers worldwide. According to 2018 global cancer statistics based on 185 countries, esophageal cancer is the seventh most common cancer and the sixth in terms of mortality globally[1]. The two major subtypes of esophageal cancer, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), differ in symptoms, geographic distribution, and etiology. ESCC is the most common subtype of esophageal cancer globally. It constitutes more than 80% of esophageal cancer cases worldwide[2-4]. In 2012, there were about 398000 new cases of ESCC, which was 7.6-fold higher than EAC cases[5]. Like many other cancer types, genetic alterations, uncontrollable proliferation, and escaping from cell death and immune-response are associated with the pathogenesis of ESCC. The high rates of local invasion and distant metastasis also contribute to the malignancy of ESCC. In fact, most patients have distant metastasis at initial diagnosis [6]. Delayed diagnosis and treatment lead to the poor prognosis. The 5-year survival rate of ESCC patients is only 20%-30%[7].

For decades, oncology studies mainly focused on dysfunction of protein-coding genes. It is only recently that the wide involvement of non-coding RNAs in cancer biology has been recognized. MicroRNAs (miRNAs) constitute one of the major families of non-coding transcripts. Typically, miRNAs are 20-24 ribonucleotides in length, and are transcribed from different genome locations, such as introns or junk DNA sequences between genes. MicroRNAs can bind to the 3'-untranslated region (UTR) of target mRNAs through imperfect base-pair complementation, and functionally promote target mRNA degradation or inhibit translation. The biogenesis of miRNAs is tightly controlled within normal cells, but mutations within miRNAs and dysregulated miRNA expression have been observed in many tumor types[8]. This highlights the possibility that miRNAs may be useful as diagnostic biomarkers. It has also been shown that miRNAs are involved in regulating multiple biological processes during cancer pathogenesis, which suggests that they may be exploited as therapeutic targets or tools[9]. In the last decades, increasing evidence supports that miRNAs have important roles in the pathophysiology of esophageal cancer[10,11]. In this review, we will summarize recent discoveries on the altered expression and functions of miRNAs in ESCC.

DYSREGULATION OF MICRORNAS IN ESCC

Genetic variations within miRNA sequence, as well as dysregulated level of miRNA, are frequently observed in multiple tumor types including ESCC[8]. Most of the genetic variations of miRNAs are due to the single nucleotide polymorphisms within miRNA coding genes (miR-SNPs)[12]. Studies in the last decades have demonstrated the epidemiological significance of miR-SNPs associated with susceptibility to ESCC in different populations. As a typical example, multiple studies reported that an SNP of miR-196a2, rs11614913 CC>TT, is associated with a reduced risk of ESCC in the Chinese Han population[13,14]. Another SNP of pro-miR-423, rs6505162 A>C, was found to be correlated with the incidence of ESCC in the Black population of South

Africa. The same study also emphasized that this allele is strongly associated with inhaling smoke from burning biomass fuel, which is a known environmental risk factor for ESCC[15]. Functionally, polymorphisms of miRNAs can affect the expression of mature miRNA oligos by changing the secondary structure and stability of pre-miRNA molecules[12,16]. Taking polymorphism of miR-196a2 as an example, the rs11614913 TT genotype is associated with reduced miR-196a expression, which weakens its oncogenic function[13]. Besides, SNPs located in miRNA binding sites within the 3'UTR of cancer-related genes are also regarded as a critical issue of miRNA dysregulation. For instance, SNP rs6573A>C in the 3'UTR of RAS-related protein (RAP1A) was found to eliminate miR-196a binding, which consequently increased RAP1A expression and promoted ESCC development[17]. Another example is that CT or TT genotype of rs2866943, which is an SNP of protein tyrosine phosphatase receptor type T (PTPRT) 3'UTR, was reported to be associated with disrupted miR-218-regulated PTPRT expression[18].

Compared with genetic alteration of miRNA by SNPs, dysregulated miRNA expression patterns in ESCC have attracted much more attention in recent years. Yang *et al*[19] performed miRNA profiling in 113 pairs of ESCC tumor and matched non-tumor tissues, and found that 39 miRNAs were dysregulated in ESCC by at least 2-fold, including 28 downregulated and 11 upregulated miRNAs. Within the list of downregulated miRNAs, some (*e.g.*, miR-133a and miR-133b) were also identified in other miRNA screening studies as possible tumor suppressors[20,21]. Dysregulation of several miRNAs was also reported to be associated with therapeutic response. For instance, miRNA profiling in ESCC tumor samples from patients receiving neoadjuvant radiochemotherapy showed that the expression of 12 miRNAs including 8 upregulated and 4 downregulated ones was altered in non-responders compared with responders[22]. In particular, high levels of miR-194 and miR-665, which were found in radiotherapy- or chemotherapy-resistant tumor samples, were correlated with a poor survival of ESCC patients, suggesting their potential as novel biomarkers for prognostic prediction[22]. Abnormal expression of miRNAs in ESCC may be due to many reasons. Genome instability is one of the underlying mechanisms. For example, Hu *et al*[23] performed global analysis of genome, mRNA, and miRNA status in 30 cases of ESCC, and demonstrated an association between bi-allelic loss in cancer genome and expression pattern change of 60 miRNAs. However, the authors also pointed out that bi-allelic loss in ESCC was infrequent, and that the relation between bi-allelic loss and miRNA dysregulation needs further validation[23]. Another piece of evidence is the location of some miRNA coding genes in the somatic copy-number alteration (SCNA) regions in cancer genome, such as miR-4448, miR-1224-3p, and miR-4707-5p in ESCC[24], which can lead to irregular mature miRNA expression. Moreover, several studies reported the correlation between dysregulated miRNA expression and abnormal epigenetic modulation in the promoter region. For example, promoter hypomethylation was found to enhance the expression of oncogenic miR-10-3p during ESCC development[25]. On the other hand, hypermethylation of CpG sites in promoter region was reported to downregulate the expression of tumor-suppressive miRNAs such as miR-375[26], miR-126[27], and miR-218[28,29] in ESCC. In addition, further studies demonstrated that miRNA expression is regulated by multiple upstream epigenetic factors, which is usually defined as "epigenetic-miRNA feedback loop"[30]. Taking miR-126 and miR-218 as examples, hypermethylation of their promoters is mediated by members of the DNA methyltransferase (DNMT) family, especially DNMT1 and DNMT3B[27,29]. Besides, epigenetic chromatin modifications also contribute to regulation of miRNA expression pattern. Koumangoye *et al*[31] reported that trimethylation of histone H3 lysine 27 (H3K27me3), which is a well-known heterochromatic modification, negatively regulated the expression of miR-31 in ESCC cells. In that study, the authors found that the SOX4/EZH2/HDAC3 co-repressor complex mediates hypermethylation of H3K27 around miR-31 promoter [31]. However, up to now, little is known about the regulatory function of other types of histone modifications on miRNA expression in ESCC. Further studies are needed to reveal the possible contribution of different histone modifications in modulating miRNA expression pattern in ESCC.

ROLES OF MICRORNAS IN TUMORIGENESIS OF ESCC

Beyond merely identifying alterations in miRNA expression patterns, researchers have become more focused on studying the functional significance of aberrantly expressed miRNAs. MiRNAs have been found to be involved in regulation of multiple biological

processes during ESCC development (Figure 1). According to the distinct roles, miRNAs can be broadly described as tumor-suppressive and oncogenic miRNAs. Generally speaking, tumor-suppressive miRNAs functionally impede tumor malignancy, and are frequently repressed in expression level in tumor cells. For example, miR-503 was reported to negatively regulate cyclin D1 and induce cell cycle arrest in G1/S phase in ESCC[32]. Similar cell proliferation-repressive miRNAs include miR-200c and miR-593*, which interfere with mitosis by targeting P21 and target polo-like kinase 1 (PLK1), respectively[33,34]. Some miRNAs also suppress development of ESCC by regulating metabolic processes, especially glucose metabolism. For instance, miR-375 exerts a metabolism-repressive role by directly inhibiting lactate dehydrogenase B, which is a key enzyme in post-glycolysis process and catalyzes pyruvate-lactate interconversion[35]. Similarly, rate-limiting enzymes in glycolysis, such as glucose-6-phosphate dehydrogenase (G6PD) and hexokinase 2 (HK2), were reported to be negatively regulated by miR-613[36] and miR-125/143 cluster in ESCC[37]. Besides, miRNAs can also exert tumor-suppressive function by inhibiting cancer stemness. For example, Li *et al*[38] found that miR-377 was frequently downregulated in ESCC tumor, and that ectopic miR-377 expression could inhibit sphere formation and tumorigenic potential of ESCC cells by directly inhibiting CD133, which is a well-known stemness biomarker in cancer. In addition, it was reported that miR-377 can target chromobox protein homolog 3 (CBX3) and contributes to maintenance of stem cell potential in ESCC[39], which further emphasized the importance of miR-377 as a critical tumor suppressor in ESCC.

Moreover, tumor-suppressive miRNAs can also promote the effect of chemoradiotherapies. A previous study by our group reported the tumor-suppressive role of miR-29c in reducing chemoresistance[40]. Overexpressing miR-29c in fluorouracil (5-FU)-resistant ESCC cell sublines increased their response to 5-FU treatment, shown as decreased cell viability and increased cell death, while miR-29c antagonist had opposite effect and could desensitize parental cells to 5-FU treatment. F-box only protein 31 (FBXO31), which has oncogenic function in ESCC[41] and lung cancer[42], was found to be one of the direct target genes of miR-29c that mediates its tumor-suppressive function. Further investigation found signal transducer and activator of transcription 5A (STAT5A) to be a transcription factor that can negatively regulate miR-29c expression in 5-FU-resistant cells[40]. This study highlighted the key role of the STAT5A/miR-29c/FBXO31 axis as a modulator of ESCC chemoresistance. By utilizing the same 5-FU-resistant cell model, Han *et al*[43] proved that miR-338-5p directly targets Id-1 (Inhibitor of DNA Binding 1) in ESCC cells to reverse chemoresistance. Interestingly, miR-338-5p was also able to attenuate cisplatin resistance and enhance radiotherapy efficiency by targeting focal adhesion protein kindlin-2 (also known as FERMT2)[44] and survivin[45], respectively. These studies suggest that miR-338-5p may be an essential tumor inhibitor.

On the other hand, onco-miRNAs are often upregulated in expression and functionally promote cancer progression by inhibiting tumor-suppressive proteins. One of the most well-studied oncogenic miRNAs in ESCC is miR-21. Several studies have reported that this miRNA is upregulated in the tumor[46-48], as well as in the serum or plasma of patients with ESCC[49-51]. Functionally, miR-21 was reported to promote cell growth and inhibit apoptosis in ESCC by activating the ERK/mitogen-activated protein kinase (MAPK) signaling cascade[52], or targeting genes including Fas ligand (FASL)[53], programmed cell death 4 (PDCD4)[54], phosphatase and tensin homolog (PTEN)[55-57], and RAS p21 protein activator 1 (RASA1)[58]. Further study also found miR-21 to play a role in maintaining cancer cell stemness in ESCC by upregulating stem cell markers such as Oct4 and Nanog and targeting TNF receptor-associated factor 4 (TRAF4)[59]. Oncogenic effects of miR-21 were also reported in breast cancer[60] and colorectal cancer[61], indicating its potential value as a pan-cancer therapeutic target. Onco-miRNAs can also interrupt cancer-related inflammation. For instance, miR-31 was found to be overexpressed in an orthotopic ESCC rat model promoted by Zn-deficiency, and functional tests revealed that miR-31 knockout abolished ESCC development and altered the expression profile of inflammation genes [62]. This effect was mediated by tumor suppressors egl-9 family hypoxia inducible factor 3 (EGLN3) and membrane bound o-acyltransferase domain containing 2 (MBOAT2)[62]. Actually, both oncogenic and tumor-suppressive functions of miR-31 were found in different cancer types[63], but knockout of miR-31 failed to show obvious genomic and metabolic instability in the rat esophagus, indicating that it might be of great value as a potential therapeutic target for ESCC[62].
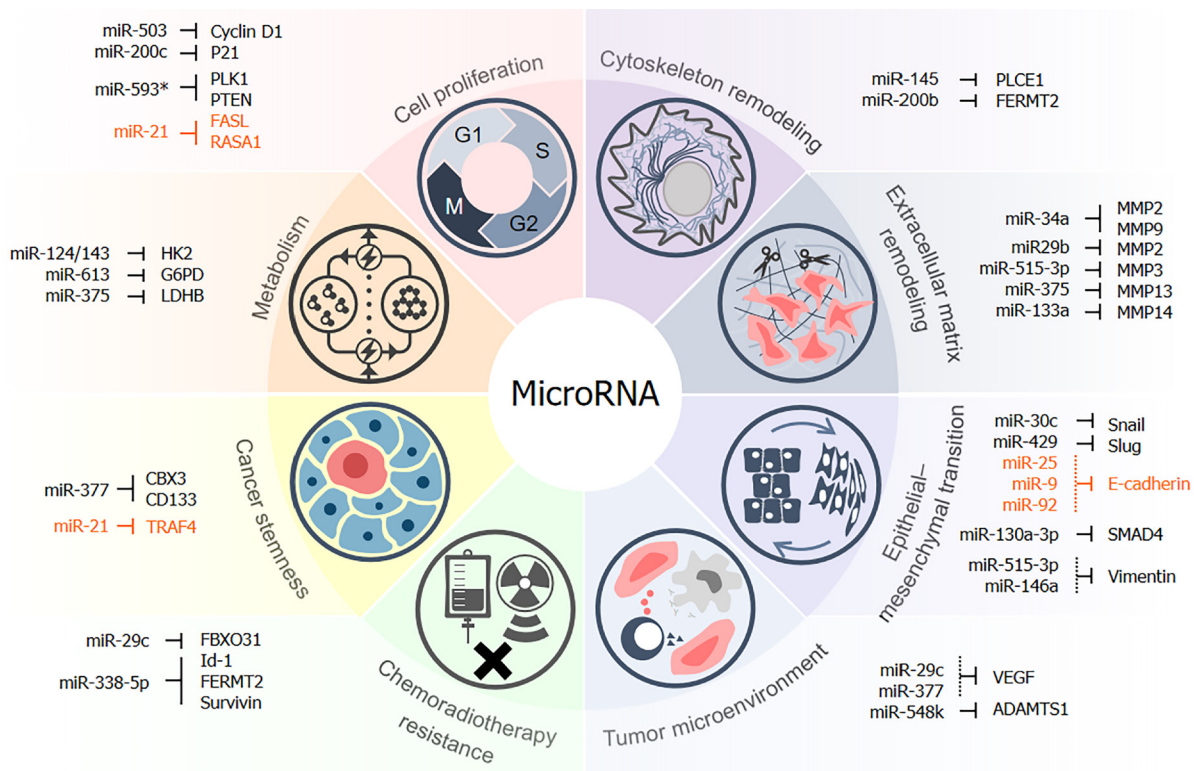
Figure 1 Multiple roles of miRNAs during development of esophageal squamous cell carcinoma. This figure summarizes the targets and regulatory functions of miRNAs in multiple biological processes during esophageal squamous cell carcinoma (ESCC) development. MicroRNAs inhibit or promote tumorigenesis and recurrence of ESCC by modulating cell proliferation, metabolism, cancer stemness, and resistance to chemo- or radiotherapy. They also regulate metastasis by targeting functional molecules involved in epithelial-mesenchymal transformation, remodeling of the cytoskeleton, extracellular matrix, and tumor microenvironment. Oncogenic miRNAs and target genes are marked in orange. PLCE1: Phospholipase C epsilon 1; VEGF: Vascular endothelial growth factor.

ROLES OF MICRORNAS IN METASTASIS OF ESCC

The high incidence of metastasis is a serious issue associated with poor prognosis of ESCC. In addition to investigating the involvement of protein-coding genes in modulating tumor metastasis, recent studies have provided much information on the regulatory roles of miRNAs (Figure 1, also summarized in Table 1). MiRNAs were reported to modulate motility of ESCC cells by regulating cytoskeleton dynamics. For instance, miR-145 was demonstrated to have a suppressive effect on metastasis of ESCC by targeting phospholipase C epsilon 1 (PLCE1)[64], which is a Ras protein associated effector that can functionally remodel actin cytoskeleton[65,66]. Another example is miR-200b, which was reported to target FERMT2[67]. Therefore, the miR-200b/FERMT2 regulatory axis can reduce cell invasiveness by modulating the structure of the cytoskeleton and blocking the formation of focal adhesion[67].

The invasiveness of cancer cells is largely dependent on remodeling of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs)[68]. Members of the MMP family are endopeptidases that catalyze the degradation of ECM components. In ESCC, highly expressed MMPs are often associated with strong malignancy and poor prognosis[69,70]. MiRNAs can modulate ESCC cell invasiveness by targeting MMPs. Yang *et al*[71] reported that a p53-downstream miRNA, miR-34a, suppressed ESCC cell migration and invasion by directly targeting and suppressing MMP2 and MMP9. Interestingly, miR-34a was also found to inhibit the expression of an upstream transcription factor of MMP2 and MMP9, namely, Yin Yang 1 (YY1), in ESCC cells, so that MMP2 and MMP9 levels were negatively regulated in ESCC[72]. Besides, MMP2 was also reported to be regulated by miR-29b in ESCC[73]. Qi *et al*[73] showed that the miR-29b/MMP2 axis inhibited cell invasion *in vitro*, as well as tumor growth in an animal model, indicating its involvement in both tumor development and metastasis. Other members of the MMP protein family, such as MMP3, MMP13, and MMP14, were found to be regulated by miR-515-3p[74], miR-375[75], and miR-133a[76], respectively.

Apart from gain of cell motility and remodeling ECM by MMPs, epithelial-mesenchymal transition (EMT) is another important process during pre-metastasis stage. Generally speaking, EMT describes the loss of epithelial cell phenotype to

Table 1 Metastasis-regulating miRNAs in esophageal squamous cell carcinoma

MicroRNA	Validated target(s)	Effect	Ref.
miR-1	Notch2	Represses proliferation, migration, and invasion	[105]
miR-9	E-cadherin	Promotes metastasis	[91]
miRNA-10b-3p	TSGA10	Promotes cell growth and metastasis	[106]
miR-17/20a	TGFBR2	Represses migration and invasion	[107]
miR-21	TGFβ	Promotes TGFβ-induced EMT	[108]
	PCD4	Promotes cell growth and invasion	[54]
	PTEN	Promotes invasion	[57]
miR-25	E-cadherin	Promotes metastasis	[93]
miR-26a and miR-144	COX2	Repress proliferation and metastasis	[109]
miR-29b	MMP2	Represses proliferation and invasion	[73]
miR-29c	VEGF	Represses angiogenesis and metastasis	[95]
miR-30c	SNAI1	Represses proliferation, EMT and invasion	[86]
miR-34a	MMP2, MMP9, and FNDC3B	Represses migration and invasion	[71]
	CD44	Represses invasion and metastasis	[110]
	Yin Yang-1	Represses migration and invasion	[72]
miR-92a	E-cadherin	Promotes lymph node metastasis	[92]
miR-92b	ITGAV	Represses invasion and metastasis	[111]
miR-106b	PTEN	Promotes invasion and metastasis	[112]
	SMAD7	Represses EMT	[84]
miR-128-3p	ZEB1	Represses EMT and metastasis	[89]
miR-130a-5p	ZEB1	Represses EMT and metastasis	[113]
miR-130-3p	SMAD4	Represses EMT, migration, and invasion	[82]
miR-133a	MMP14, FSCN1	Represses cell invasion	[76]
miR-145	PLCE1	Represses proliferation and metastasis	[64]
miR-146a	Vimentin	Represses tumor invasion	[85]
miR-150	ZEB1	Represses EMT and metastasis	[90]
miR-200b	Kindlin-2	Represses focal adhesion formation and invasion	[67]
miR-218	BMI1	Represses proliferation and metastasis	[114]
miR-339-5p	TSPAN15	Represses metastasis	[115]
miR-375	SHOX2	Represses invasion and metastasis	[116]
	MMP13	Represses migration and invasion	[75]
miR-377	CD133 and VEGF	Represses tumor initiation and progression	[38]
miR-424-5p	SMAD7	Represses EMT, migration and invasion	[83]
miR-429	Slug	Represses migration and invasion	[87]
miR-515-3p	MMP3 and Vimentin	Represses invasion and metastasis	[74]
miR-548k	ADAMTS1	Promotes lymph node metastasis	[96]
miR-630	Slug	Represses invasion and metastasis	[117]
miR-644a	PITX2	Repressing aggressiveness and stem cell-like phenotype	[118]
miR-655	TGFBR2 and ZEB1	Represses EMT	[88]
miR-1290	SCAI	Promotes proliferation and metastasis	[119]
miR-4324	FAK	Represses EMT	[120]

| miR-6775-3p | MAGE-A family proteins | Represses invasion and metastasis | [121] |

TGF-β: Transforming growth factor β; PTEN: Phosphatase and tensin homolog; PLCE1: Phospholipase C epsilon 1; VEGF: Vascular endothelial growth factor.

assume a mesenchymal cell phenotype. It is considered to be a transitional process with a spectrum of stages, such as losing cell polarity and cell-cell junction, enhancing migratory property and invasiveness, and acquiring resistance to anoikis[77-79]. The EMT process is controlled by many growth factors, but the dominant inducer is considered to be transforming growth factor β (TGF-β)[80]. TGF-β stimulates Smad and MAPK signaling, thereby inducing a series of genetic events during EMT. Activation of SNAIL, ZEB, and TWIST transcription factor families is the initiation step. These transcription factors silence the gene expression of tight junction molecules of epithelial cells such as E-cadherin, tight junction protein 1 (TJP1), and zonula occludens 1 (ZO-1), and translationally activate the expression of mesenchymal marker molecules such as N-cadherin and vimentin[81]. Up to now, several miRNAs have been found to regulate the EMT process by directly targeting these related elements during metastasis of ESCC. For example, miR-130a-3p was reported to suppress EMT in ESCC by targeting SMAD4, which is one of the downstream molecules of the TGFβ signaling pathway[82]. Similarly, SMAD7 can be regulated by miR-424-5p[83] and miR-106b[84]. The miR-515-3p/vimentin regulatory axis can reverse EMT and negatively regulate ESCC metastasis[74]. Another miRNA that targets vimentin, namely, miR-146a, was also reported to suppress ESCC invasion by disrupting fibronectin membrane assembly[85]. Two members of the SNAIL transcription factors family, Snail and Slug, were found to be regulated by miR-30c[86] and miR-429[87], respectively. The transcription factor ZEB1 is negatively regulated in ESCC by miR-655 [88], miR-128-3p[89], and miR-150[90]. On the contrary, miR-25, miR-9, and miR-92 act as oncogenic miRNAs that can promote EMT and ESCC metastasis by targeting E-cadherin[91-93].

Metastasis is affected by biochemical and cellular components surrounding tumor cells, which constitute the tumor microenvironment (TME). MiRNAs are functionally involved in the interaction between ESCC cells and the TME. On the one hand, the expression pattern of miRNAs in ESCC cells can be modulated by the TME, especially under stressful conditions such as hypoxia[94]. On the other hand, miRNAs are also known to regulate the crosstalk between ESCC cells and surrounding cancer-associated cells. In particular, several studies have found that miRNAs modulate the expression or secretion of vascular endothelial growth factor (VEGF). For instance, one of the previous studies in our group reported that miR-29c could suppress VEGF expression in cancer-associated fibroblasts, and the expression of miR-29c was in turn regulated by insulin-like growth factor 2 (IGF2) secreted by ESCC cells, suggesting the existence of a IGF2/miR-29c/VEGF regulatory loop which can promote ESCC distant metastasis[95]. Another study performed by our group found that VEGF was also targeted and inhibited by miR-377 in ESCC cells, so that the miR-377/VEGF axis had a negative impact on ESCC metastasis by downregulating angiogenesis[38]. In addition, Zhang *et al*[96] demonstrated that miR-548k directly inhibited metalloproteinase ADAMTS1, which acted as a chaperone and blocked intrinsic VEGFC. In addition, high level of miR-548k in ESCC cells facilitated VEGFC secretion. As a consequence, the miR-548k/ADAMTS1/VEGFC axis promoted lymph node metastasis by activating VEGFR3 in lymphatic endothelial cells[96].

THERAPEUTIC POTENTIAL OF MICRORNAS IN ESCC

In view of increasing evidence proving that miRNAs have important regulatory roles in cancer, miRNAs are considered as a pool of ideal therapeutic targets for developing novel treatment strategies. To be specific, treatment with synthetic antagonists or inhibitors may specifically neutralize and block the endogenous activity of oncogenic miRNAs. This is proposed as "mRNA suppression therapy". As for tumor-suppressive miRNAs, "miRNA replacement therapy", which is defined as restoring the loss of tumor-suppressive miRNA and enhancing the post-transcriptional repression using miRNA mimicking oligonucleotides or agomirs, is appropriate[97,98]. As a type of gene therapy, miRNA-based therapy has numerous advantages. First, the small molecular weight of miRNA oligonucleotides[98] renders them easier to deliver *in vivo*

than plasmids or viral-based gene therapies. Second, miRNAs act as network regulators and can mediate silencing of multiple target genes or pathways simultaneously. This is believed to induce enhanced anti-cancer effects, compared to conventional therapies such as chemoradiotherapy and single-targeting inhibitors[97,99]. Third, miRNAs have lower toxicity compared with DNA or protein-based therapies because they are endogenously produced by cells[98,99]. Therefore, novel miRNA-based cancer therapies have become an exciting development in recent years.

Current studies on miRNAs in ESCC mostly focus on the identification of clinical correlation and novel targeting axes[100-102]. Less is known about the therapeutic potential of specific miRNAs in treatment. Isozaki *et al*[103] showed that subcutaneous injection of miR-375 suppressed ectopic ESCC tumor growth *in vivo*. Another miRNA, miR-27a, had similar tumor-suppressive effect on ESCC tumor growth *in vivo* after direct injection into implanted tumor[104]. Most recently, three miRNAs including miR-377, miR-29c, and miR-515-3p proved to have therapeutic potential through systemic delivery *via* intravenous injection in mouse models[38,40,74]. Specifically, systemically administered miR-377 was able to suppress the growth of subcutaneous ESCC tumor xenografts and lung metastasis in a mouse model[38]. Systemic delivery of miR-29c produced a synergistic effect in inhibiting tumor growth when combined with 5-FU treatment[40]. Oligonucleotide mimicking miR-515-3p inhibited lung metastasis of ESCC in nude mice[74].

CONCLUSION

Studies in recent years have underscored the salient involvement of miRNAs in cellular events and human disease. A number of dysregulated miRNAs have been identified in multiple cancer types and nominated as potential novel cancer biomarkers. We now have a better understanding of how miRNAs can simultaneously regulate multiple target genes, and thereby functionally behave as oncogenes or tumor suppressors during cancer development and progression. Most importantly, researchers have discovered the therapeutic potential of miRNAs as novel targets or agents. In summary, studies in ESCC confirmed that miRNAs form a complex regulatory network in modulating tumor progression and malignancy. However, related investigations on the clinical application of miRNAs are still scarce. Therefore, future in-depth research on regulatory mechanisms of miRNAs and further validation of their therapeutic efficacy will allow us to better exploit this ubiquitous class of small molecules.

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