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MicroRNAs: A novel signature in the metastasis of esophageal squamous cell carcinoma

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Abstract

Esophageal squamous cell carcinoma (ESCC) is a malignant epithelial tumor, characterized by squamous cell differentiation, it is the sixth leading cause of cancer-related deaths globally. The increased mortality rate of ESCC patients is predominantly due to the advanced stage of the disease when discovered, coupled with higher risk of metastasis, which is an exceedingly malignant characteristic of cancer, frequently leading to a high mortality rate. Unfortunately, there is currently no specific and effective marker to predict and treat metastasis in ESCC. MicroRNAs (miRNAs) are a class of small non-coding RNA molecules, approximately 22 nucleotides in length. miRNAs are vital in modulating gene expression and serve pivotal regulatory roles in the occurrence, progression, and prognosis of cancer. Here, we have examined the literature to highlight the intimate correlations between miRNAs and ESCC metastasis, and show that ESCC metastasis is predominantly regulated or regulated by genetic and epigenetic factors. This review proposes a potential role for miRNAs as diagnostic and therapeutic biomarkers for metastasis in ESCC metastasis, with the ultimate aim of reducing the mortality rate among patients with ESCC.

Key Words: MicroRNAs; Esophageal squamous cell carcinoma; Metastasis; Signaling pathway; Epigenetics mechanism

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Core Tip: Esophageal squamous cell carcinoma (ESCC) is the sixth leading cause of cancer-related deaths globally predominantly due to metastasis. MicroRNAs (miRNAs), acting either as tumor suppressors or oncogenes, play crucial roles in the development and progression of tumors. We herein discussed the intimate correlations between miRNAs and ESCC metastasis, predominantly associated with genetic and epigenetic regulatory, and aimed to propose the potential role of miRNAs as diagnostic and therapeutic biomarkers for ESCC metastasis.

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INTRODUCTION

Esophageal cancer, the eighth most prevalent cancer worldwide, ranks as the sixth leading cause of cancer-related deaths [1]. There are two major histological types of esophageal cancer, which include esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC)[2]. Despite significant advancements in the treatment of ESCC treatment, both morbidity and mortality rates continue to rise annually. This is largely due to the advanced stage of the ESCC at diagnosis, combined with an increased propensity for metastasis[3,4]. When ESCC cells become metastatic and acquire the ability to invade surrounding tissues and enter the bloodstream or lymphatic system, they can travel to distant organs such as the liver, lungs, gastrointestinal tract, pancreas, and bones to establish secondary tumors[5].

Cancer metastasis is a complex process that encompasses a series of stages including local invasion (involving epithelial-to-mesenchymal transition, EMT), survival and entry into the bloodstream or lymphatic system (intravasation), exit from the bloodstream or lymphatic systems (extravasation), and proliferation at a new location (colonization). Examining this intricate process can offer valuable insights into the development of novel therapeutic strategies for cancer treatment. Numerous studies have demonstrated that a variety of signaling pathways, primarily receptor tyrosine kinase (RTK), transforming growth factor- β (TGF- β), Wnt/ β -catenin, and the interleukin (IL)-6/Stat3 pathways, can partake in the process of cancer metastasis process[6-10]. Furthermore, evidence suggests that abnormally expressed microRNAs (miRNAs) can impact cancer metastasis by modulating these signaling pathways[7-10].

MiRNAs are endogenous short non-coding RNAs (approximately 22 nucleotides) that can regulate gene expression by inhibiting mRNA transcription or by promoting mRNA degradation through sequence-specific binding in the 3'-untranslated region (3' -UTR). They often function as master regulators of gene expression and play crucial roles in the development and progression of tumors by acting as either as tumor suppressors or oncogenes[11]. This review focuses on the role and mechanism of miRNAs in facilitating ESCC metastasis of ESCC, and also aims to highlight the potential use of miRNAs as diagnostic and therapeutic biomarkers in the management of ESCC metastasis.

THE ROLE OF MIRNAS IN THE METASTASIS OF ESCC METASTASIS

Tissues from cancer patients, cancer cell lines, and xenograft models are commonly employed to investigate the occurrence and mechanisms of cancer cell metastasis. In the tissues of cancer patients, lymph node metastasis (LNM), vessel invasion (VI), lymph node invasion (LNI), lymphatic invasion (LI), tumor nodes metastasis stage (TNM), and depth of invasion (DI), are often used to evaluate the metastatic characteristics of the cancer. In cancer cells, the cell invasion, migration and EMT often are commonly used to evaluate the ability of tumor cells to metastasize. In xenograft models, the location and quantity of tumor distribution are used to identify the metastatic potential of tumors. Thus, we assess the role and underlying mechanism of miRNA in the metastasis of ESCC by examining its metastatic behavior in tissues, cells, and xenograft models (Tables 1 and 2)[12-15].

Numerous studies have found that miRNAs, acting either as tumor suppressors or oncogenes, play crucial roles in the development and progression of tumors[13]. As tumor suppressors, some miRNAs can inhibit tumor occurrence and development by targeting genes that have the potential to promote oncogenesis and progression[16]. Conversely, as oncogenes, some miRNAs, often called oncomiRs, can promote tumor malignancy by suppressing the expression of genes that normally prevent cells from becoming cancerous[17]. Multiple studies over the past decade have highlighted the importance of miRNAs also acting as suppressors or oncogenes in ESCC metastasis.

MiRNAs act as tumor suppressors in ESCC metastasis

There is a significant reduction in the expression levels of tumor-suppressive miRNAs in both tissues and cells associated with ESCC. These miRNAs substantially influence the malignancy and metastatic potential of ESCC malignancy, including ESCC metastasis (Table 1). For example, miRNAs like miR-138, miR-193a-5p, miR-206, miR-451, and miR-718 are markedly decreased in ESCC-associated tissues, and these decreases are significantly correlated with LNM and the TNM stage[18-22]. Furthermore, the reduced expression of miRNAs, including but not limited to miR-100, miR-124, miR-125b, miR-126, miR-128-3p, and miR-129, are not only positively associated with ESCC metastasis in ESCC patient tissues,

Table 1 MicroRNAs act as suppressor in esophageal squamous cell carcinoma metastasis

miRNA	Metastasis studies in different sources			Ref.
Suppressor	Tissue	Cell	Xenograft	
miR-1	LNM, VI, TNM, advanced C stage	I		[148]
miR-10a	LNM, C stage	I, M, EMT	Tumor Me	[29]
miR-100	LNM	I, M		[24]
miR-100-5p	DI, LI, T stage			[149]
miR-101	LNM	I, M	Liver Me	[30]
miR-10527-5p	LNM, T stage, LI	I, M, EMT, LI		[9]
miR-107	NS (LNM)	I, M		[152]
miR-122		I, M, EMT		[116]
miR-124	LNM, Me	I, M		[25]
miR-124-3p	LNM, DI, DM, TNM	I, M, EMT		[31,156]
miR-125a-5p		I, M		[157]
miR-125b	LNM, TNM	I, M, EMT		[26]
miR-1254	VI, P stage			[159]
miR-126	LNM, TNM	I, M		[27]
miR-128-3p	LNM	I, M, EMT		[23]
miR-129	TNM	I, M		[28,143]
miR-129-2	LNM, DM, advance clinical TNM	I, M		[164]
miR-129-2-3p		I, M		[166]
miR-1294	LNM, LI, VI	I, M		[84]
miR-130a-5p		I, M		[169]
miR-133a	LNM, P stage	I, M		[170]
miR-133b		I, M, EMT		[102]
miR-134	LNM, TNM	I, M		[32]
miR-136-5p	LNM, TNM	I, M		[168]
miR-137		I, M, EMT		[174]
miR-138	LNM, TNM			[18]
miR-140-3p	LNM, DM, TNM	I, EMT		[176]
miR-140	Me	I, M, EMT		[178]
miR-143	LNM, DI, VI, TNM	I, M		[13]
miR-143-3p		I, M		[180]
miR-144	Me, TNM	I, M	Liver Me	[33]
miR-145	LNM, DI, TNM	I, M, EMT		[182, 183]
miR-145-3p		I, M		[185]
miR-145-5p		I, M, EMT		[187]
miR-146a	NS (N stage), TNM	I, M, EMT		[189]
miR1469	LNM, DI			[191]
miR-148a		I, M, EMT		[193]
miR-149-5p		I, M		[10]
miR-150	LNM, LI, VI, TNM	I, M, EMT		[195]

miR-150-3p		I, M		[197]
miR-153		I, M, EMT		[94]
miR-181a-5p		I, M	DLA Me	[199]
miR-185	NS (LNM, DM, TNM)	I, M	Lung Me	[15]
miR-186-5p	NS (LNM), DM	I, M		[43]
miR-193a-5p	LNM, TNM			[19]
miR-193b		I, M		[104]
miR-195	LNM, VI, TNM	I, M		[202]
miR-198		I, M		[204]
miR-202	LNM	M		[206]
miR203	LNM, TNM	I, M, EMT	Lung Me	[34,81]
miR-203a-3p		I, M, EMT		[209]
miR-204-5p	LNM, DI	I, M, EMT		[210]
miR-205	LNM	I, M, EMT		[93,213]
miR206	LNM, TNM			[20]
miR-212-3p		I		[215]
miR-214	LNM, tumor stage	I, M, EMT		[216,217]
miR-214-3p		I, M		[219]
miR-216a/b	LNM, TNM			[220]
miR-217		I, M		[80]
miR-218	LNM, TNM	I, M		[221]
miR-26a	LNM, C stages	I, M	Liver Me	[33,223]
miR-27a	LNM	I		[103]
miR-27b-3p	LNM, TNM	I, M, EMT		[225]
miR-29b	LNM	I, M		[88]
miR-29c	MPI			[228]
miR-30a	LNM	I, M		[148]
miR-30b	NS (N stage)	I, M, EMT		[44]
miR30a-3p	LNM	I, M, EMT		[148]
miR-30c	T, N, M, stage	I, EMT		[95]
miR-30d	LNM, advanced TNM	I, M		[150]
miR-30e-5		I, M		[126]
miR-301a		I, M		[151]
miR-323a-3p		I, M	Lung Me	[153]
miR-326		I, M		[154]
miR-328		I, M	Liver Me	[155]
miR-33a-5p	LNM, TNM	I, M		[111]
miR-335-5p		I, M, EMT		[158]
miR-338-3p		I, M, EMT		[101]
miR-338-5p		I, M	Lung Me	[160]
miR-34a	LNM, TNM	I, M, EMT	Liver Me	[36,161]
miR-340-5p	LNM, TNM	I, M		[162]
miR-3612		I, M		[163]

miR-365		I, EMT		[165]
miR-370	LNМ, tumor invasion			[167]
miR-370-3p	LNМ, TNM	I, M		[168]
miR-375	LNМ, LVI, DM, TNM	I, M, EMT	Tumor mobility, I	[37,38]
miR-377	LNМ, TNM	I, M		[171]
miR378	LNМ, TNM	I, M,		[172]
miR-378a-3p		I, M		[11]
miR-422a		I, M		[173]
miR-429	LNМ, TNM	I, M, EMT		[175]
miR-4324	LNМ, DI	I, M, EMT		[175]
miR-433-3p		I, M		[177]
miR-4429	LNМ, TNM	I, M, EMT		[114]
miR-449a-5p	LNМ, TNM			[179]
miR-451	LNМ, TNM			[21]
miR-455-3p	LNМ	I		[181]
miR-455-5p	LNМ	I, M		[184]
miR-4766-5p		I, M, EMT		[186]
miR-485-5p	NS (LNМ), TNM	I, M, EMT		[188]
miR-486-5p		I		[190]
miR-490-3p		I, M, EMT		[192]
miR-493		I, M		[145]
miR-497-5p		I, M		[194]
miR-498	NS (LNМ, DM)	I, M		[196]
miR-508-3p		I, M		[198]
miR-515-3p	Me	I, M, EMT	Lung ME	[12]
miR-516b	LNМ, DI, advanced TNM	I, M, EMT		[200]
miR-518b	LNМ	I		[201]
miR-542-3p	NS (metastasis)	I, M		[47]
miR-574-3p	LNМ, DI, TNM	I, M		[50]
miR-590	LNМ, TNM	I, M, EMT		[51]
miR-595		I, M		[203]
miR-599		I, M, EMT		[205]
miR-615-5p	LNМ, advanced TNM	I, M		[207]
miR-620		I, M, EMT	Lung ME	[208]
miR-622	N stage, TNM	I, M, EMT		[138]
miR-625	LNМ, DM, advanced TNM	I		[211,212]
miR-630	LNМ, P stage	I, M, EMT		[214]
miR-652	LNМ, TNM	I, M	Multiple ME	[39]
miR-670-3p		I, M		[139]
miR-671-5p		I, M, EMT		[218]
miR-718	LNМ, TNM			[22]
miR-765		I, M		[133]
miR-874-3p	LNМ, C stage	I, M		[14]

miR-942-5p	TNM, C stage	I, M, EMT	[222]
miR-98	LNM, TNM	I, M	[216]
miR-99a		I, M, EMT	[224]
let-7	LNM		[226]
let-7a	TNM	I, M, EMT	LNM [227]
let-7b-5p		I, M	[40]

LNM: Lymph node metastasis; VI: Vessel invasion; LVI: Lymphatic vessel invasion; LI: Lymphatic invasion; TNM: Tumor nodes metastasis stage; C stage: Clinical stage; P stage: Pathological stage; DI: Depth of invasion; DM: Distant metastasis; I: Invasion; M: Migration; EMT: Epithelial-mesenchymal transition; Me: Metastasis; DLA Me: Dilated lymphatic arteries metastasis; Multiple Me: Brain, bone, adrenal gland, lung, kidney, liver metastasis; MPI: Muscularis propria invasion.

but also promotes ESCC cell invasion, migration, and/or EMT processes[23-28]. Several studies have confirmed the inhibitory effects of some miRNAs on ESCC metastasis at the tissue, cell, and animal model levels, including miR-10a, miR-101, miR-124-3p, miR-134, miR-144, miR-203, miR-26a, miR-34a, miR-375, miR-515-3p, miR-652, and let-7b-5p[12,29-40]. Intriguingly, our research reveals that certain miRNAs, including miR-107, miR-146a, miR-185, miR-186-5p, miR-30b, miR-485-5p, miR-498, and miR-542-3p, among others, have minimal effects on ESCC metastasis in tissue samples. However, they can significantly suppress ESCC cell invasion and migration and have a considerable impact on metastasis in xenograft models[15,41-47].

The role of these miRNAs as tumor suppressors heavily relies on the tumor-promoting activities of their target genes. For instance, the significant downregulation of miR-101 in ESCC tissues correlates strongly with LNM. Moreover, miR-101 can suppress tumor metastasis both *in vitro* (in xenograft models) and *in vivo* (in ESCC cells). Notably, the inhibitory effect of miR-101 in ESCC migration and invasion is reversed *via* activation of its target genes, COX-2, MALAT1, or EZH2. These genes are identified as playing crucial roles in promoting tumor development and progression, although not exclusively in ESCC[30,48,49]. Another notable suppressor miRNA is miR-574-3p, whose reduced expression is negatively associated with LNM, TNM stage, and invasion depth. Inhibition of miR-574-3p promotes migration and invasion of ESCC cells *in vitro*. However, the knockdown of its target genes, FAM3C or MAPK1, can lessen the increase in migration and invasion observed following treatment with the miR-574-3p inhibitor. The majority of studies suggest that both FAM3C and MAPK1 promote malignant tumor development, including proliferation, invasion, and migration[50].

MiRNAs act as oncogenes in ESCC metastasis

Similar to cancer-suppressive miRNAs, oncomiRs have also been identified as promoters of ESCC tissue infiltration, as well as ESCC cell invasion, migration, and epithelial-mesenchymal transition (EMT) in ESCC (Table 2)[51-77]. The increased expressions of miR-10b-3p, miR-106b-5p, miR-25, miR-320b, miR-602, miR-9, and miR-99b/Let-7e/miR-125a not only show significant correlations with metastasis in ESCC patients, but these miRNAs are also known to increase the invasion, migration, and/or EMT of ESCC cells. Additionally, these miRNAs can promote the metastasis of transplanted tumors, primarily to the lungs[52-58]. Other miRNAs, including miR-1269, miR-17-5p, miR-18a-5p, miR-25-3p, miR-301, miR-373, miR-483-5p, miR-766-3p, and others, display oncogenic roles in metastasis, affecting both ESCC tissues and cells [59-67]. In addition, some miRNAs including but not limited to miR-103a-2-5p, miR-105-5p, miR-130b, miR-1323, miR-19b-3p, miR-196a, miR-23b-3p, and miR-301b display potent pro-metastatic functions, primarily pertaining to cellular activity[68-75].

The oncogenic potential of oncomiRs also hinges on their target genes, which typically act as tumor suppressors by preventing cells from becoming malignant. For instance, PTEN, a well-known tumor suppressor, is downregulated by several oncomiRs, including miR-1323, miR-25-3p, miR-301, miR-624-3p, miR-92a-3p, and others. The expression of these oncomiRs in ESCC tissues and/or cells is significantly upregulated and further research has shown that the effects of PTEN overexpression, including inhibition of cell migration and invasion, effects that can be partially reversed by the aforementioned oncomiRs[62,63,71,76,77]. Programmed cell death 4 (PDCD4), which functions as a tumor suppressor in ESCC, is a direct target of miR-320b. Exosomal miR-320b has been shown to promote LNM and lymphangiogenesis both *in vitro* and *in vivo*. Additional *in vitro* studies have confirmed that re-expression of PDCD4 can not only rescue its downregulation, but can also counteract lymphangiogenesis and LN metastasis mediated by exosomes with high levels of miR-320b[53].

THE GENETIC MECHANISM OF MIRNAS ON ESCC METASTASIS

Research evidence indicates that both genetic and epigenetic regulatory mechanisms of miRNAs play a crucial role in tumorigenesis and development, including metastasis in ESCC[54,78].

Tumor metastasis-associated genes (TMAGs) represent a category of genes that serve a vital function in the process of metastasis. This group includes metastasis suppressor genes (MSGs), and metastasis promoting genes (MPGs). MSGs and MPGs are primarily involved in various signaling pathways, including the RTK, transforming growth factor- β (TGF- β), Wnt/ β -catenin, and interleukin (IL)-6/Stat3 pathways. They also target specific and key factors, including ZEB1/2,

Table 2 MicroRNAs act as oncogene in esophageal squamous cell carcinoma metastasis

miRNA	Metastasis studies in different sources			Ref.
oncomiR	Tissue	Cell	Xenograft	
miR-10b-3p	LNM, C stages	I, M, EMT	Lung, Liver Bone Me	[56,57]
miR-10b	NS (LNM)	I, M		[229]
miR-103a-2-5p		I		[68]
miR-105-5p		I, M		[69]
miR-106b-5p	TNM	I, M, EMT	Lung Me	[52]
miR-1179	Me TNM	I		[232]
miR-1269	LNM, TNM	I, M		[59]
miR-1290	LNM, tumor invasion, TNM	I, M		[113]
miR-130b		I, M		[70]
miR1323		I, M		[71]
miR-135b-5p	LNM	I, M		[236]
MiR-140-5p		I, M, EMT		[92]
miR-142-3p	LNM			[237]
miR-1470		M		[239]
miR-17-5p	LNM, TNM, DI	I, M		[60,240]
miR-17a	LNM, C stage			[242]
miR-18a	LNM, TNM	I, M		[61,244]
miR-181b-5p	Advance TNM	M		[107]
miR-181c-3p	DI, TNM			[246]
miR-19b	LNM, C stage			[242]
miR-19b-3p		I, M		[72]
miR-196a		I, M		[74]
miR-196a-3p		M		[74]
miR-20b-5p	LNM, C stage	I, M		[249]
miR200a		I, M		[87]
miR-21 ¹	LNM, LI, VI, TNM	I, M, EMT		[282]
miR-221		I, M		[251]
miR-224	NR (LNM), TNM	I, M		[252]
miR-224-5p		I, M, EMT		[100]
miR-23a	NS	I, M	Lung Me	[140,254]
miR-23b-3p		I, M, EMT		[73]
MiR-23b-5p	Me	I, M	Lung Me	[257]
miR-25	LNM, TNM	I, M, EMT	Lung Me	[58]
miR-25-3p	LNM, TNM	I, M		[62]
miR-30d-5p	LNM, TNM, DI	I, M, EMT		[91,230]
miR-301	LNM, TNM	I, M		[63]
miR-301a-3p	LNM, tumor invasion, TNM			[231]
miR-301b		I, M		[75]
miR-31	N, M stage, TNM	I, M, EMT		[233]
miR-320b	LNM	I, M, EMT		[53]

miR-330-3p		I, M		[234]
miR-3656		I, M		[235]
miR-373	LNM, TNM	I, M		[64]
miR-425		I, M		[119]
miR-4286		I, M		[238]
miR-4443	TNM	I		[60]
miR-452-5p	LNM, DM	I, M		[241]
miR-483-3p		I, M, EMT		[243]
miR-483-5p	LNM, TNM	I, M		[65,66]
miR-506	LNM, TNM			[245]
miR-5692b	DI, TNM			[246]
miR-602	LNM, TNM	I, M	Lung, liver, bone, adrenal gland Me	[54]
miR-612	LNM, metastasis	I, M		[247]
miR-624-3p		I, M, EMT		[76]
miR-675-3p		I, M, EMT		[248]
miR-766-3p	LNM, TNM	I, M		[67]
miR-875-5p		I, M		[250]
miR-9	LNM	I, M, EMT	Lung Me	[85]
miR-92a	LNM, LNI	I, M		[86]
miR-92a-3p		I, M		[77]
miR-92b-3p		I, M		[253]
miR-935	Me			[255]
miR93		I, M		[256]

¹NS (plasma).

LNM: Lymph node metastasis; VI: Vessel invasion; LNI: Lymph node invasion; LI: Lymphatic invasion; TNM: Tumor nodes metastasis stage; C stage: Clinical stage; DI: Depth of invasion; I: Invasion; M: Migration; Me: Metastasis; EMT: Epithelial-mesenchymal transition.

Snail1/2, E-cadherin, N-cadherin, C-myc, Vimentin, and MMPs, all of which are recognized as markers of tumor metastasis. Substantial evidence indicates that miRNAs exert their function on ESCC metastasis by targeting tumor suppressor genes or oncogenes, which belonging to the above-mentioned signaling pathways and their downstream signaling molecules[79].

MiRNAs directly target markers of tumor metastasis

Research indicates that miRNAs can regulate the metastasis of ESCC by directly targeting markers associated with tumor metastasis (Figures 1 and 2), including ZEB1/2, Snail1/2, E-cadherin, N-cadherin, C-myc, Vimentin, and members of the Matrix Metalloproteinase family (MMPs), *etc.*[12,26,78,80-84].

E-cadherin, predominantly found in epithelial tissues, is crucial for maintaining cell adhesion, polarity, and tissue architecture. Within the context of cancer metastasis, E-cadherin acts as a tumor suppressor. It serves as a critical biomarker and regulator of tumor metastasis, and its altered expression may indicate cancer progression. miR-9, miR-25, miR-92a, and miR-200a have been found to either directly or partially mediate tumor metastasis (including LNM, EMT, invasion, and migration) through E-cadherin[83,85-87].

The MMP family consists of enzymes mainly responsible for the degradation of extracellular matrix (ECM) components. In cancer, MMPs promote tumor growth, invasion, and metastasis by breaking down the ECM barriers, thereby facilitating cancer cell migration. Furthermore, they are involved in angiogenesis, a process of new blood vessel formation critical for tumor growth, survival, and metastasis. MMP2, MMP3, MMP9, and MMP13 are targets for miR-29b, miR-515-3p, miR-34a, and miR-375, respectively. These miRNAs are wholly or partly involved in ESCC metastasis[12,88-90].

Additionally, several transcription factors (TFs), particularly ZEB1/2 and Snail1/2, play critical roles in tumor metastasis. Their primary function is to downregulate the expression of epithelial markers (like E-cadherin) and upregulate the expression of mesenchymal markers (like N-cadherin and Vimentin), thereby driving the process of EMT process[81,82,91,92]. ZEB2, is a direct target of miR-140 and miR-205, and it reverses the effects of these miRNAs on EMT, migration, and invasion of ESCC cells[78,93]. miR-30c, miR-30d-5p, miR-203, and miR-153 are known to directly target

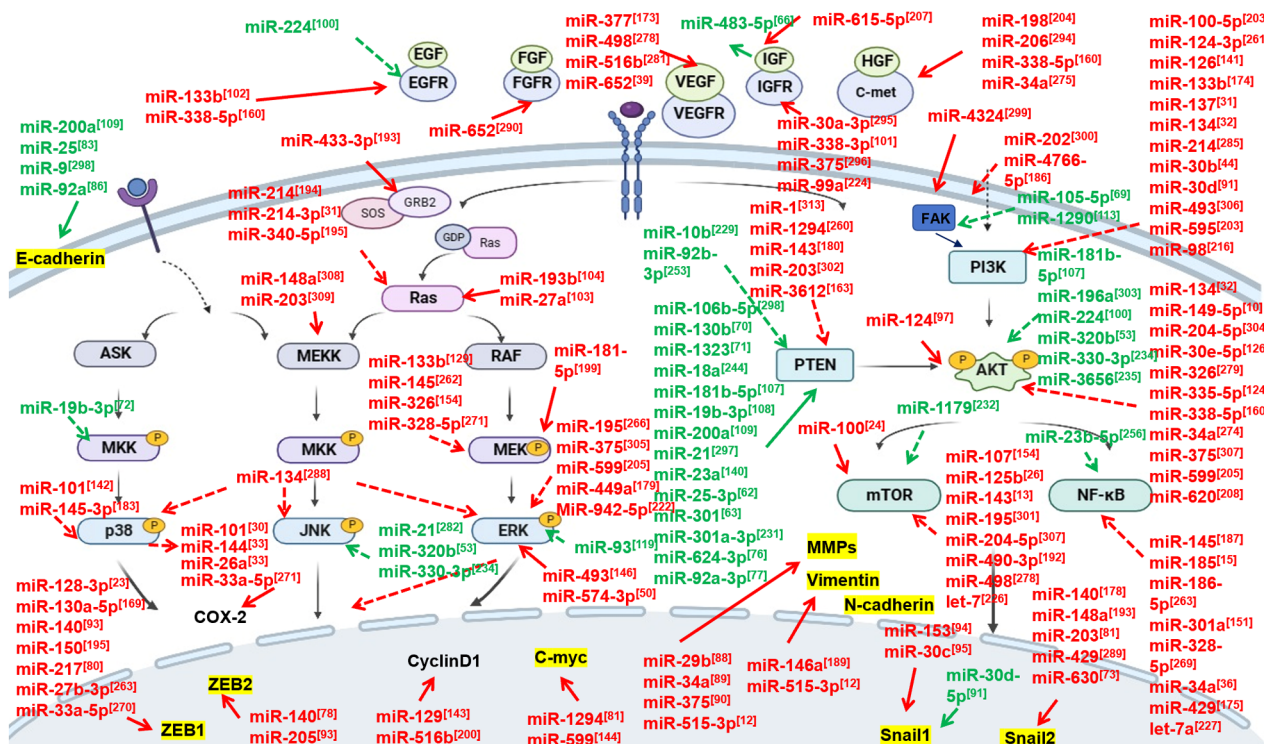


Figure 1 MicroRNAs targeting receptor tyrosine kinase signaling pathway in esophageal squamous cell carcinoma metastasis. The receptor tyrosine kinases (RTKs) signaling pathway effect many signals, including epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF) and their receptors, as well as intracellular signaling pathways, such as mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K). The dysregulation of miRNAs on the RTK signaling pathway is play important roles in ESCC metastasis[10,12,13,15,23,24,26,30-33,36,39,44,50,53,62,63,66,69,70-73,76-78,80,81,83,85,86,88-91,93,95,97,100-104,107-109,112,117,123,125,128,140-144,146,151,154,160,163,169,174,175,178-180,185-187,189,192,193,195,199,200,203-205,207,208,216,222,224,226,227,229,231,232,234,235,244,253,257,260-265,266,269-271,273,274,278,279,281,282,285,289-309,311]. ZEB1/2, Snail1/2, E-cadherin, N-cadherin, C-myc, Vimentin and MMPs are specific target and key factors for esophageal squamous cell carcinoma metastasis. "Green" and "Red" indicated oncomiRs and tumor-suppressive miRNAs, respectively. The dashed arrow and solid arrow indicated direct targeting effect and indirect targeting effect, respectively.

Snail1, a critical regulator of metastasis. Overexpression of these miRNAs, either individually or in combination, can counteract metastatic behaviors in whole or in part, thereby presenting a potential therapeutic strategy for mitigating cancer metastasis[82,91,94,95].

MiRNAs targeting RTK signaling pathway in ESCC metastasis

The RTKs signaling pathway serves as a central conduit for communication within and between cells, regulating a multitude of cellular functions including cell growth, differentiation, invasion, and migration[7]. This pathway comes into effect when specific signals, typically growth factors or hormones such as epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF), bind to their corresponding RTK on the cell surface. Upon activation, the RTK acts as a platform for various intracellular signaling pathways, but primarily, the mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K) pathways. MAPK signaling, a common and highly conserved pathway, primarily includes extracellular signaling-associated kinases (ERK1/2), Jun amino-terminal kinases (JNK), and p38-MAPK. The PI3K/Akt pathway can be irregularly activated *via* several mechanisms, including various genomic alterations such as mutations in PTEN, Akt, and mTOR mechanism[7,71,96,97]. Dysregulation of the RTK signaling pathway is implicated in the malignant progression of ESCC, including metastasis[4,98,99].

Numerous miRNAs have been identified as crucial regulators in ESCC metastasis by targeting RTK signaling, suggesting a critical crosstalk between miRNAs and the RTK signaling is necessary for ESCC metastasis (Figure 1)[100,101]. Certain miRNAs exert control over ESCC metastasis by directly interacting with growth factors or growth factor receptors, such as RTKs, subsequently regulating their downstream signaling pathways and molecules. For instance, miR-133b can repress ESCC cell invasion and metastasis by targeting the EGFR, which inhibits the PI3K/Akt signaling pathway and curbs the expression of downstream molecules such as N-cadherin, MMP-2, MMP-9, and E-cadherin[102]. Similarly, the downregulation of miR-338-3p, whose target is the IGF1R, activates the Raf/MEK/ERK pathway and affects the expression levels of E-cadherin, N-cadherin, and vimentin, and thus promoting ESCC cell invasion, migration, and EMT in ESCC[101]. Certain miRNAs are known to directly target the downstream signaling of the RTK pathway to influence ESCC metastasis. For example, miR-27a and miR-193b, functioning as tumor suppressors, are downregulated in ESCC and suppress cell migration and invasion *via* the direct regulation of KRAS, which is linked to the MAPK/ERK signaling pathway[103,104]. The tumor suppressor protein PTEN is able to dephosphorylate Akt to lessen its activation, thus blocking all downstream signaling events controlled by Akt and acting as a negative regulator of PI3K. Numerous

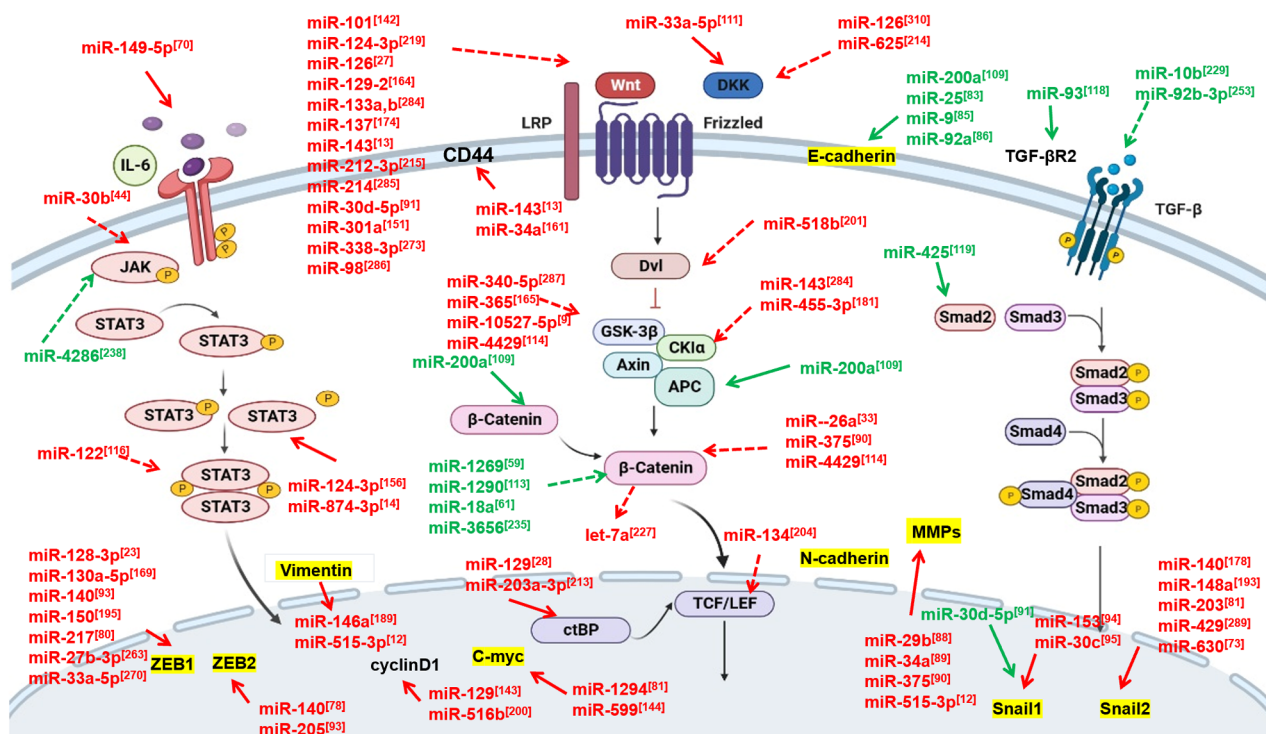


Figure 2 MicroRNAs targeting the pathways of Wnt/ β -catenin, IL6/STAT3, and TGF- β in esophageal squamous cell carcinoma metastasis.

In esophageal squamous cell carcinoma (ESCC), miRNAs serve as crucial effector to Wnt/ β -catenin, IL-6/STAT3 and TGF- β signalings, which is consistently involved in cancer metastasis[9, 12, 14, 23, 27, 28, 33, 44, 59, 61, 70, 73, 78, 80, 81, 83, 85, 86, 88-91, 93-95, 109, 111, 113, 114, 116, 118, 119, 142-144, 151, 156, 161, 164, 165, 169, 174, 178, 181, 189, 193, 195, 200, 201, 204, 209, 211, 215, 219, 227, 229, 235, 238, 253, 263, 270, 273, 284-287, 289, 310]. Wnt/ β -catenin signaling includes Wnts, receptors (such as Frizzled, FZD and low-density lipoprotein receptor-related proteins, LRP), Dishevelled (Dsh/Dvl), β -catenin, glycogen synthase kinase 3 β (GSK-3 β), Axin, APC (adenomatous polyposis coli). IL-6 is known to activate the downstream JAK/STAT3 signaling, and canonical TGF- β pathway is mediated by SMAD2, SMAD3, and SMAD4. ZEB1/2, Snail1/2, E-cadherin, N-cadherin, C-myc, Vimentin and MMPs are specific target and key factors for ESCC metastasis. "Green" and "Red" indicated oncomiRs and tumor-suppressive miRNAs, respectively. The dashed arrow indicated direct targeting effect and solid arrow indicated indirect targeting effect, respectively.

miRNAs, including but not limited to miR-106b-5p, miR-130b, miR-1323, miR-18a, miR-181b-5p, miR-19b-3p, and miR-200a, exhibit a conspicuous overexpression and a negative association with LNM and/or TNM classification in ESCC. This overexpression fosters the progression of ESCC by facilitating cell invasion, metastasis, and/or EMT[52,71,105-109]. Furthermore, most of miRNAs whether indirectly or directly targeting PTEN can decrease the anticancer effect of PTEN, and promote the invasion and metastasis of ESCC[70,71,108].

miRNAs targeting the Wnt/ β -catenin signaling pathway in ESCC metastasis

The Wnt/ β -catenin signaling pathway assumes a pivotal role in various physiological processes, such as cell proliferation, differentiation, migration, and apoptosis. This signaling pathway consists of several components, including Wnts, receptors (such as Frizzled, FZD and low-density lipoprotein receptor-related proteins, LRP), Dishevelled (Dsh/Dvl), β -catenin, glycogen synthase kinase 3 β (GSK-3 β), Axin, and APC (adenomatous polyposis coli). Research has shown that the Wnt/ β -catenin signaling pathway is intricately linked with tumor development, partly as a significant contributor to the onset and metastasis of ESCC, including metastasis[98,110].

Studies suggest that miRNAs can directly or indirectly modulate various components of the Wnt/ β -catenin pathway, thereby either activating or inhibiting ESCC metastasis (Figure 2). For instance, miR-33a-5p, which exhibits diminished expression in ESCC tissues and correlates with higher TNM staging and LNM, can directly target Dickkopf-related protein 1 (DKK1) thereby influencing the metastasis of ESCC *via* the Wnt/ β -catenin pathway[111]. Additionally, a majority of miRNAs associated with ESCC were found to indirectly target molecules associated with the Wnt/ β -catenin pathway. One such example is miR-340, which is significantly downregulated in ESCC tissues and is highly correlated with LNM and TNM stages. phosphoserine aminotransferase 1 (PSAT1), *via* the GSK3 β / β -catenin/Cyclin D1 pathway, was identified as a direct target of miR-340, although it can also partially counteracts the miR-340 mediated inhibition of viability, invasion, and EMT in ESCC cells[112]. Beyond miR-200a, Numerous miRNAs, including suppressor miRNAs and oncomiRs, have been found to indirectly regulate β -catenin, playing a critical role in ESCC metastasis. For example, both of miR-1269 and miR-1290 act as oncomiRs, and are upregulated in ESCC tissues and cell lines. They are associated with positive LNM and/or advanced TNM stage, and their overexpression promotes the malignant transformation of ESCC cells, including migration and invasion, by directly targeting SRY-box transcription factor 6, interactive with β -catenin (SOX6)[59,113]. Furthermore, the downregulation of miR-4429 has been shown to foster ESCC cell invasion and migration by partially regulating β -catenin through the direct targeting of SRXN1[114].

MiRNAs targeting IL6/STAT3 signaling pathway in ESCC metastasis

IL-6, a pleiotropic cytokine, is implicated in a diverse array of pathological processes including chronic inflammation, autoimmune diseases, and tumors, and other diseases. IL-6 is known to activate the downstream JAK/STAT3 signaling pathway, a hallmark of many malignant tumors, thereby contributing to tumor onset and, progression, and metastasis [115]. In ESCC, miRNAs serve as crucial effectors to the IL-6/STAT3 pathway, which is consistently involved in cancer metastasis (Figure 2). One such instance is miR-149-5p, a direct target of IL-6. The overexpression of miR-149-5p suppresses IL-6 expression at both the mRNA and protein levels, thereby inhibiting the invasion and migration of ESCC cells [10]. Similarly, miR-874-3p, is found downregulated in ESCC tissues and cell lines and exhibits a statistically significant association with LNM and clinical stage. Its overexpression markedly suppresses migration and invasion in ESCC cells by directly targeting STAT3 [14]. Intriguingly, several miRNAs, such as miR-30b and miR-122, have been found to modulate ESCC cell invasion, migration, and EMT by indirectly regulating JAK and STAT3, respectively [44,116].

MiRNAs targeting transforming growth factor- β signaling pathway in ESCC metastasis

The transforming growth factor- β (TGF- β) signaling pathway is a classical signal transduction pathway, encompassing both the conventional Smad-dependent pathway and the non-Smad-dependent pathways. The canonical pathway is mediated through the transcription factors SMAD2, SMAD3, and SMAD4, while the non-canonical pathways include MAPK, nuclear factor (NF)- κ B, and PI3K/AKT/mTOR signaling, *etc* [8]. TGF- β signaling has been identified as a pivotal regulator in the modulation of the progression and metastasis of various types of cancer, including in ESCC. In the context of ESCC, our discussion primarily focuses on the role of the canonical pathway. TGF- β , induces EMT activation via the TGF- β /Smad signaling pathway [117].

In recent years, there has been a surge in investigations demonstrating that miRNAs play a pivotal role in the metastasis of ESCC through the modulation of the TGF- β pathway (Figure 2). For instance, miR-93-5p is highly expressed in ESCC cells and is known to enhance cell proliferation, migration, and invasion, while simultaneously suppressing apoptosis in ESCC cells. It achieves these effects by targeting and downregulating the expression of TGF β R2 [118]. Similarly, miR-425 is overexpressed in both ESCC tissues and plasma, and positively influences cell migration and invasion. It functions as an oncogene by specifically targeting the 3'-untranslated region (3'-UTR) of SMAD2, thereby modulating its expression and subsequent cellular behavior [119].

EPIGENETICS EFFECTS ON MIRNAS IN ESCC METASTASIS

Epigenetics refers to the study of heritable changes in gene expression that occur without a change in DNA sequence. Epigenetic changes occur in extreme cases and contain several regulatory mechanisms, including noncoding RNAs (ncRNAs), DNA methylation, heterochromatin, polycomb and trithorax proteins and 3D genome architecture [120]. In recent years, accumulating evidences suggest that different expression patterns of ncRNA play increasingly important roles in cancer. ncRNAs are made of distinct classes, including noncoding RNA (lncRNA), miRNA, circRNA, rRNA, tRNA, and so on [121-123]. Among them, more exciting progress has been made in the study of the role and mechanism of lncRNA, circRNA and the impact of DNA methylation of miRNAs in tumor processes (Table 3).

The crosstalk between lncRNA and miRNA in ESCC metastasis

lncRNA are ncRNAs with lengths > 200 nucleotides. They represent diverse types of RNA molecules with limited or no protein-coding capability, and different biological functions depending on their subcellular location. Cytoplasmic lncRNAs exhibit vital roles in cancer, often acting as tumor suppressors or oncogenes through the modulation of miRNAs [124]. Recently, accumulated evidences has demonstrated that lncRNAs are involved in ESCC metastasis by acting as competing endogenous RNAs (ceRNAs) that “sponge” miRNAs to block their function, and then, up-regulate the downstream genes [125]. For example, lncRNA DLEU2, is up-regulated in EC tissues and associated with poor prognosis. The overexpression of this lncRNA DLEU2 increased the proliferation, migration and invasion abilities of ESCC by suppressing miR-30e-5p and then directly targeting E2F7. Another lncRNA, lncRNA-IUR up-regulates PTEN by “sponging” miR-21 and acting as a tumor suppressor in ESCC metastasis [126]. Antisense lncRNAs, a special type of lncRNA, are antisense RNAs with partial exon overlap with forward genes. They can also regulate gene expression by competitively binding to certain miRNA in ESCC metastasis, such as PSMA3-AS1, ZFAS1, TTN-AS1, NCK1-AS1, THAP9-AS1, FAM83A-AS1, OIP5AS1, DDX11-AS1, SLC2A1-AS1, and MAFG-AS1 [48,91,124,127-133]. MAFG-AS1 is significantly up-regulated in ESCC tissues and cell lines, and accelerates ESCC cell proliferation, migration, invasion and aerobic glycolysis by competitively adsorbing miR-765, a negative modulator of PDX1 expression of PDX1. Overexpression of lncRNA HAND2-AS1 its overexpression inhibited cell proliferation, migration, and invasion by downregulation of miRNA-21 in ESCC cells [133,134].

The crosstalk between circRNA and miRNA in ESCC metastasis

CircRNAs also act as miRNA “sponges” and comprise a large class of endogenous non-coding RNA with covalently closed loops. CircRNAs have independent functions from the linear transcripts that are transcribed from identical genes [135]. Recently, it was demonstrated that numerous circRNAs are differentially expressed in ESCC, and their dysfunction is linked to ESCC metastasis. The typical circRNAs contain circHIPK3, circ2646, circOGDH, circ_0001273, circABC10, circ_0001946, circLARP4, circ_0007624, circFoxo3 [25,71,100,113,136-140]. circ-OGDH was upregulated in ESCC cells and its inhibition repressed cell proliferation, migration, and invasion, and reduced miR-615-5p-induced cellular glutamine

Table 3 The epigenetic regulation on microRNAs in esophageal squamous cell carcinoma metastasis

miRNA (Suppressor)	Epigenetic regulation	Ref.
miR-101	~/ <i>lncMALAT1</i> <i>lncXIST</i> /~/EZH2 PSMA3-AS1/~/EZH2	[48,49,142]
miR-122	LINC01410/~/PKM2	[259]
miR-129	<i>lncNEAT1</i> /~/CtBP2; <i>lnc XIST</i> /~/CCND1	[28,145]
miR-130a-5p	CCL18-induced <i>lncHOTAIR</i> /~/ZEB1	[169]
miR-133b	TTN-AS1/~/FSCN1 NCK1-AS1/~/ENPEP <i>lncKCNQ1OT1</i> /~/EGFRTHAP9-AS1/~/SOX4	[102,128,129,261]
miR-145	<i>lncROR</i> /~/FSCN1	[262]
miR-140-5p	<i>lncSNHG16</i> /~/ZEB1	[92]
miR-186-5p	<i>lncSNHG6</i> /~/HIF-1 α	[264]
miR-195	<i>lncSNHG1</i> /~/Cdc42	[265,266]
miR203	circ_0008717/~/Slug circPRMT5/~/	[35,267]
miR206	circ_0008726/~/	[20]
miR-214	FAM83A-AS1/~/CDC25B LINC00963/~/RAB14	[130,268]
miR-30a	OIP5AS1/~/VOPPI	[131]
miR-30d-5p	DDX11-AS1/~/SNAI1	[91]
miR-30e-5p	<i>lncDLEU2</i> /~/E2F7	[126]
miR-301a	<i>lncGAS5</i> /~/CXCR4	[151]
miR-328	<i>lncLOC146880</i> /~/FSCN1	[269]
miR-33a-5p	<i>lncDANCR</i> /~/ZEB1 <i>lncCASC15</i> /~/PTGS2	[270,271]
miR-335-5p	<i>lncTHAP9-AS1</i> /~/SGMS2	[124]
miR-338-3p	<i>lncBRAF</i> /~/IGF1R <i>lncSNHG17</i> /~/SOX4	[101,273]
miR-34a	<i>lncMIR31HG</i> /~/c-Met	[274]
miR-340-5p	LINC00662/~/HOXB2	[162]
miR-378a-3p	SLC2A1-AS1/~/Glut1	[132]
miR-498	<i>lncTUG1</i> /~/Cdc42 AFAP1-AS1/~/VEGFA <i>lncTUG1</i> /~/XBP1	[46,294,278]
miR-508-3p	<i>lncPCAT-1</i> /~/ANXA10	[198]
miR-516b	<i>lncJPX</i> /~/VEGFA	[281]
miR-765	MAFG-AS1/~/PDX1	[135]
oncomiR		
miR-18a-5p	<i>lncPART1</i> /~/SOX6	[61]
miR-21	<i>lncRNA-IUR</i> /~/PTEN	[134,282]

	HAND2-AS1/~	
miR-1290	circ_0086414/~ /SPARCL1circ_0001946/~ /SOX6	[113,283]
miR1323	circLARP4/~ /PTEN	[71]
miR-224	circ_0007624/~ /CPEB3	[100]
miR-23a	circFoxo3/~ /miR-23a	[140]
miR-125a-5p	lncHOTAIR/~ /HK2	[258]
miR-124	circHIPK3/~ / AKT3	[25,136]
	circ2646/~ /PLP2	
miR-1294	circ_0004370/~ /LASP1	[260]
miR-140-3p	circNTRK2/~ /NRIP1	[176,222]
	circ0087378/~ /E2F3	
miR-198	circLPAR3/~ /C-met	[204]
miR-217	circZDHHHC5/~ /ZEB1	[80]
miR-27b-3p	circLONP2/~ /ZEB1	[263]
miR-3612	circ_0006948/~ /LASP1	[163]
miR-422a	circUBAP2/~ /Rab10	[173]
miR-433-3p	circ_0023984/~ /REV3L	[177]
miR-4766-5p	circPDE3B/~ /LAMA1	[186]
miR-490-3p	circ_0006948/~ /HMGA2	[192]
miR-497-5p	circ-AGFG1/~ /SLC1A5	[194]
miR-595	circNRIP1/~ /SEMA4D	[203]
miR-599	circHIPK3/~ /c-myc	[144,205]
	circ_0030018/~ /ENAH	
miR-615-5p	circOGDH/~ /PDX1	[139]
miR-622	circ_0001273/~ /SLC1A5	[138]
miR-670-3p	circABCB10/~	[139]
miR-874-3p	circ0000977/~	[272]
miR-126	DNMT1/~ /ADAM9	[141]
miR-137	CTCF/(DNA methylation)/~ /EZH2+PXN	[174]
miR-107	circSFMBT2/~ /SLC1A5	[275,276]
	LINC00152/~ /Rab10	
miR-149-5p	lnc GACAT3/~ /FOXM1	[10,277]
	circ_0000654/~ /IL-6	
miR-326	LINC01711/~ /FSCN1	[154,279]
	circATIC/~ /ID1	
miR-377	LncNEAT1/~ /E2F3	[171,280]
	circ_0072088/~ /VEGF	
miR-493	LINC00324/~ /MAPK1	[145,146]
	circFIG4/~ /E2F3	
miR-124-3p	lncZFAS1/~ /STAT3	[31,156]
	DNMT1/~ /BCAT1 ~ /EZH2/H3K27me3	
miR-10b-3p	DNA methylation; Hypoxia	[56,57]
miR-320b	DGCR8+METTL3- N6 -methyladenosine (m6A)/~	[53]

miR-483-5p	Igf2 methylation gene promoter/Igf2/~ / ARHGDI A	[66]
miR-602	CpG hypomethylation/~	[54]
miR-515-3p	Promoter hypomethylation	[12]

metabolism to regulate PDX1 expression[137]. circLARP4 expression was observably downregulated in ESCC, and its overexpression circLARP4 restrained cell proliferation and migration in ESCC cell. And circLARP4 was also able to act as a sponge for miR-1323 and negatively modulated miR-1323 *via* the PTEN/PI3K/ AKT pathway in ESCC[71].

The crosstalk between DNA methylation and miRNA in ESCC metastasis

DNA methylation is a form of DNA chemical modification that can alter genetic expression without altering the DNA sequence. It is a common occurrence in malignancies and is implicated in tumor initiation and progress[31]. Methylation of the CPG island, usually located in the promoter region of genes, its methylation also plays an important role in ESCC metastasis by up-regulating expression of various miRNAs, including miR-483-5p, miR-602[54,66]. miR-483-5p was overexpressed in preoperative serum and cancer tissues and is significantly correlated with the TNM stage and LNM. Low methylation of the Igf2 gene promoter region may promote the expression of Igf2, which directly affects the expression of miR-483-5p and its target genes, including Rho GDP dissociation inhibitor α , activated leukocyte cell adhesion molecule, and suppressor of cytokine signaling 3[66]. miR-602 expression was also increased in human ESCC and significantly correlated with LNM and TNM stage, and showed positive effects on ESCC cell invasion and migration by targeting Fork head box (FOX)K2 (FOXK2). Additionally, direct evidence has shown that the overexpression of miR-602 in the ESCC tissues was correlated with promoter hypomethylation and that demethylation of the promoter genes could upregulate the expression of miR-602[54]. DNA methyltransferases (DNMTs) often are involved in DNA methylation, thereby often modifying gene function through the regulation of gene expression. In ESCC, miR-124-3p shows a high correlation with TNM stage and can directly targets the mRNA 3'UTR region of BCAT1. The expression of miR-124-3p itself is regulated by hypermethylation-silencing regulation mediated by DNA methyltransferase 1 (DNMT1) [31]. The DNMT1/miR-126 epigenetic circuit contributes to ESCC proliferation and migration *via* ADAM9/EGFR/ AKT signaling; ADAM9 has been identified as a key target of miR-126 and ectopic expression of miR-126 or silencing of ADAM9 reduced ESCC cell proliferation and migration by inhibiting EGFR-AKT signaling; Downregulation of miR-126 was due to promoter hypermethylation of its host gene Egfl7, and DNMT1 was aberrantly upregulated in ESCC and is responsible for the hypermethylation of Egfl7; Intriguingly, overexpression of miR-126 suppressed DNMT1, was suppressed by overexpression of miR-126, indicating the existence of a regulatory feedback circuit[141].

Multiple epigenetic regulatory mechanisms and miRNA in ESCC metastasis

A more complex and dynamic epigenetic regulatory network of miRNA has been shown to be positively involved in ESCC metastasis. For example, one lncRNA, circRNA can “sponge” several miRNAs. lncXIST, serves as oncogene and is significantly upregulated in ESCC tissues and cells, and is significantly associated with a poor prognosis in ESCC patients. Downregulation of lncXIST can inhibit ESCC cell proliferation, migration and invasion by elevating expression of miR-101 and decreasing the expression of EZH2. Downregulation of lncXIST also functions by inhibiting CCND1 expression *via* “sponging” of miR-129-5p[142,143]. circHIPK3 is highly expressed in ESCC tissues and cell lines and is associated with advanced TNM stage, LNM and tumor size. circHIPK3 can promote ESCC cell proliferation, invasion, and migration by modulating the miR-599/c-MYC axis, as well as *via* the regulation of ESCC cell proliferation, migration, and EMT by absorbing miR-124 *via* target AKT3[25,144]. Not more than that, the same miRNA can be regulated by different epigenetic mechanisms. For example, miR-493-5p, is dramatically downregulated in ESCC tissues and cells due to “sponging” from both circFIG4 and linc00324, both of which modulated ESCC progression including ESCC cell invasion and migration *via* targeting the miR-493-5p/E2F3 and miR-493-5p/MAPK1 axis, respectively[145-147]. Another example, miR-124-3p, was found to have positive correlations with ESCC invasion and migration, as well as EMT *via* the regulation of lncZFAS1 and through DNA methylation[31,127].

CONCLUSION

ESCC is a cancer that originates from the squamous epithelial cells lining the esophagus. It is notorious for its aggressive progression and high propensity for metastasis or dispersion to other regions of the body. In ESCC, metastasis contributes to approximately 90% of cancer-associated fatalities, primarily due to the resulting disruption of digestive function, complications arising from metastasis, an overall deterioration of health, and poor prognostic outcomes. The treatment of metastatic ESCC is intricate, possibly necessitating a combination of therapeutic modalities including surgery, radiation therapy, chemotherapy, and targeted treatments. However, these interventions frequently carry their own array of side effects and complications, and their efficacy can be restricted in the face of metastatic progression. As such, deciphering the mechanisms that underpin metastasis and pioneering effective measures for its prevention and treatment are pivotal objectives in ESCC research.

The metastatic process of ESCC is influenced by a variety of factors, including tumor characteristics, host factors, microenvironmental elements, as well as genetic and epigenetic variations. Growing evidence suggests that miRNA signatures represent an epigenetic mechanism that can profoundly impact various stages of metastasis by regulating gene

expression. This regulation occurs through multiple signaling pathways, primarily including the RTK, TGF- β , Wnt/ β -catenin, and IL6/Stat3 pathways. Given their regulatory capabilities, miRNAs have surfaced as potential novel diagnostic, prognostic, and therapeutic markers for ESCC metastasis. In their capacity as diagnostic and prognostic markers, certain miRNAs are consistently overexpressed or underexpressed in ESCC tissues. These miRNAs show negative or positive associations with characteristics of metastasis, such as tumor invasive depth, lymphatic/vascular invasion, lymph node metastasis, and distant metastasis. Intriguingly, specific miRNAs have even been linked to metastatic potential, being associated with the propensity of a tumor to metastasize, the severity of metastasis, and the likely sites of metastatic spread. As therapeutic targets, miRNAs that suppress ESCC metastasis can be restored through the application of miRNA mimics, while those promoting ESCC metastasis can be suppressed using anti-miRNA molecules. The regulation of miRNAs is also influenced by other epigenetic factors, including lncRNAs, circRNAs, and DNA methylation. Additionally, miRNAs play a crucial role in molding the tumor microenvironment, affecting angiogenesis, and modulating the immune response to tumors. Given their central roles, miRNAs hold significant potential as metastatic biomarkers for both the diagnosis and therapy of ESCC.

Recent advancements in miRNA-based therapy revolve around two principal tactics: the suppression of oncogenic miRNAs to reinstate the activity of their targeted tumor suppressor genes, and the augmentation of tumor suppressor miRNAs to dampen the expression of the oncogenes they regulate. Although they are typically downregulated in cancers, enhancing the expression of tumor suppressor miRNAs can correct the aberrant overexpression of oncogenes they normally control. Additionally, the artificial introduction of tumor suppressor miRNAs can replenish deficient miRNA levels, thereby targeting and disrupting cellular pathways that contribute to tumor development and metastasis. At present, a variety of therapeutic strategies capitalizing on miRNA mimics or inhibitors are being intensively investigated. One such example is MRX34, a liposome-encapsulated miR-34 mimic that has been introduced into clinical trials for the treatment of patients with primary liver cancer or other malignancies involving the liver. Despite the significant therapeutic potential of miRNAs, several challenges loom must be addressed before their comprehensive integration into clinical practice. One of the foremost challenges in the field of miRNA therapy lies in the potential for off-target effects. These occur when the treatment unintentionally affects genes that have no direct connection to the disease being addressed, leading to unintended and potentially detrimental side effects. Such inadvertent gene regulation can be attributed to the inherent sequence similarities among miRNAs. Moreover, the expansive binding capacity of miRNAs exacerbates this issue; a single miRNA molecule may attach to multiple, disparate mRNAs, thereby simultaneously regulating a host of unrelated genes. This promiscuity in target selection underscores the complexity of achieving precise therapeutic outcomes with miRNA-based interventions. Another substantial challenge in miRNA therapy is achieving effective delivery and ensuring stability. The targeted transport of miRNAs or their inhibitors to designated tissues or cells without loss of function is an ongoing obstacle. Additionally, miRNAs are inherently unstable in the bloodstream due to rapid degradation by nucleases, which calls for the development of sophisticated systems capable of safeguarding these fragile therapeutic agents during delivery. Furthermore, the administration of exogenous miRNA or miRNA mimics may elicit an immune response that could undermine the safety and efficacy of the treatment. Determining the optimal dosage of miRNAs is equally critical, as incorrect dosing could compromise the therapeutic balance, impacting both treatment outcomes and patient well-being. Finally, there are still limitations in accurately predicting miRNA targets, understanding miRNA-mRNA interactions, and quantifying miRNA expression levels, all of which can impede the development of miRNA-based therapies.

As research advances and technology evolves, scientists and healthcare professionals are pioneering methods to navigate the complexities of miRNA therapy. For instance, the integration of large-scale functional screenings, sophisticated bioinformatics analyses, and strategic chemical modifications are being employed to attenuate off-target effects and bolster specificity. Moreover, an array of innovative delivery mechanisms, including nanocarriers, viral vectors, and exosome-based systems, are being utilized to shield miRNAs from enzymatic degradation and to ensure precise tissue-specific targeting with elevated efficiency. Enhancements in the structural design of miRNAs to assist in evasion of immune detection stand as a testament to the proactive measures being taken to preclude unsolicited immune responses. Additionally, gaining a nuanced understanding of the complex miRNA-mRNA interactions, as well as the epigenetic mechanisms at play, is necessary in order to illuminate new potential applications for miRNA-based interventions.

In summary, persistent explorations in the field of miRNA research are unlocking significant opportunities for improving diagnostic accuracy, refining prognostic predictions, and advancing the therapeutic strategies employed in the battle against cancer. With steadfast commitment to research, the future of miRNAs in medicine appears to be both promising and profound.

FOOTNOTES

Co-first authors: Qi-Ying Wei and Feng Jin.

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