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DISEASES OF THE ESOPHAGUS

Review article

Distinctive microRNAs in esophageal tumor: early diagnosis, prognosis judgment, and tumor treatment

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SUMMARY. **Esophageal tumor (ET) is aggressive and has poor prognosis. Although the incidence of ET has been reduced by the changing tumor profile, the 5-year survival and mortality rate of ET has not significantly changed, and the outlook has remained bleak. Therefore, new molecular markers for early diagnosis and prognosis judgment are urgently required. In recent years, tumor has been widely regarded as genetic disease along with epigenetic abnormalities. DNA methylation, histone deacetylation, chromatin remodeling, gene imprinting, and noncoding RNA regulation are the major parts of epigenetic regulation. Mounting evidence exists that miRNAs (microRNA), a class of small, endogenous, and non-protein-coding RNAs, provide a novel tool for early clinical diagnosis, prognosis judgment, and gene therapy of ET. In this review, we provide a general overview of the connection between miRNA profiles and their target genes. We also describe in detail in ET from the aspect of clinical insights, the potential application of miRNAs as biomarkers, potential diagnostic and therapeutic tools.**

KEY WORDS: **early diagnosis, esophageal tumor, microRNA, prognosis judgment, tumor treatment.**

INTRODUCTION

Esophageal tumor (ET) is one of the most lethal malignancies of the digestive tract according to the latest estimation, as well as the sixth most common malignant tumor and the seventh leading cause of cancer-related deaths in the world.[1] Cancer survival tends to be poorer in ET, most likely because of a combination of a late stage at diagnosis and limited access to timely and standard treatment, highlighting the need to identify biomarkers for early detection and prognostic classification. MiRNAs were previously thought to have little importance because they were not directly translated into a protein like their coding counterparts; however, it was recently found that miRNAs do, in fact, have a more important role than previously thought.[2] It has been demonstrated that all the known processes involved in cancer, including apoptosis, proliferation, survival, and metastasis, are regulated by this small regulatory noncoding RNAs.[3] At present, miRNAs has become the rising stars in cancer genetics.

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MIRNA BIOGENESIS

MicroRNAs are small noncoding RNAs involved in posttranscriptional regulation of mRNAs stability and protein translation. Their genes are evolutionarily conserved, and may be located either within the introns or exons of protein-coding genes (70%) or in intergenic areas (30%).[3] MiRNAs are preferentially transcribed by polymerase II into long primary molecules (pri-miRNA) that are subsequently processed in the nucleus by the enzyme Drosha to become ~70 nt long precursor strands (pre-miRNA).[4–6] Then, pre-miRNA is exported by exportin 5 to the cytoplasm, where it is bound to the miR-induced silencing complex (miR-ISC), which is composed of the transactivation-responsive RNA-binding protein and Argonaute (Ago).[7–9] First, Ago cleaves the pre-miRNA 12 nt from its 3' end (forming Ago-cleaved precursor miRNA), and then the cytoplasmic RNase III Dicer cuts pre-miRNA into an 18- to 22-nt long

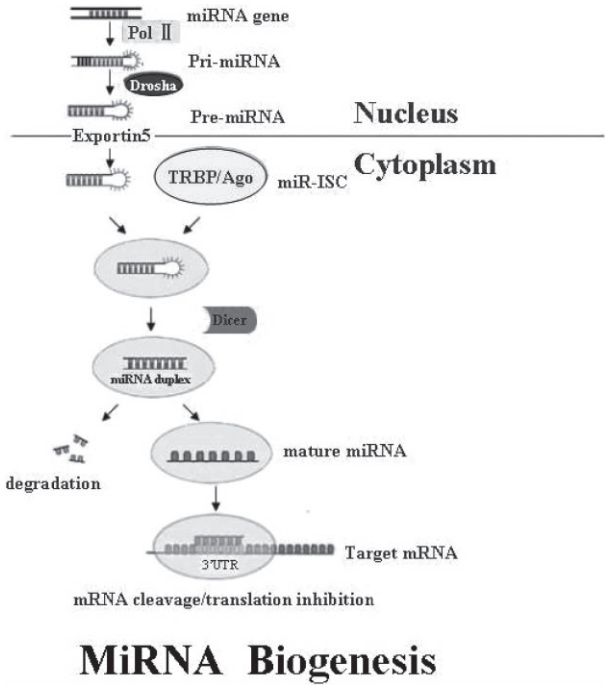

Fig. 1 MiRNA biogenesis: MiRNA is transcribed by RNA polymerase II into long pri-miRNA, which are recognized and cleaved in the nucleus by the RNase III enzyme Drosha, resulting in a hairpin precursor form called pre-miRNA. Then, pre-miRNA is exported from the nucleus to the cytoplasm by exportin 5 and is further processed by another RNase enzyme called Dicer, which produces a transient 19–24-nt duplex. Only mature miRNA is incorporated into miR-ISC, and induces mRNA translational repression by complimentarily binding in the 3'UTR of target mRNAs. ^{3}Ago, Argonaute; miR-ISC, miR-induced silencing complex; TRBP, transactivation-responsive RNA-binding protein.

RNA duplex. While the mature strand is retained in miR-ISC, the other strand is removed and degraded. The mature ~22 nt strand recognizes complementary sequences in the 3'UTR (untranslated region) of target mRNAs, and guides the miR-ISC to repress gene expression by inhibiting translation and inducing mRNA cleavage (Fig. 1).[10,11]

MIRNA PROFILES AND ESOPHAGUS TUMORIGENESIS

ET is characterized by the striking geographic variation throughout the world. Esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) are the most common histopathological types of ET. In Asian countries, including Japan and China, ESCC is the most common type, but adenocarcinoma is almost uniquely histopathological features in the west. Before 2009, miRNAs' researches mainly focus on expression profiles of ET. Many sophisticated studies showed that different diseases of esophagus have different expression profiles of miRNAs. Now, it is generally accepted that upregulated miRNAs usually have oncogenic roles to be categorized in oncogenic miRNAs (oncomiRs),[12–17] while downregulated miRNAs may have tumor-suppressive effects to be categorized in tumor-suppressive miRNAs (ts-miRs)[18–22] in cancer.

MIRNA PROFILES IN BE AND EAC

BE (Barrett's esophageal) is the principle identifiable precursor to EAC, as it can progress through varying grades of dysplasia to EAC. Fassan and colleagues identified miRNA signature of Barrett's carcinogenesis consisting of an increased expression of six miRNAs and a reduced expression of seven miRNAs.[23] Another research demonstrates feasibility of miRNAs to discriminate BE patients with and without dysplasia with reasonable clinical accuracy.[24] The expression of miR-143 in the normal and neosquamous epithelium from patients with Barrett's esophagus who underwent Argon plasma coagulation was higher than in normal squamous epithelium controls, suggesting that miR-143 could promote a Barrett's epithelium gene expression pattern and might have a role in the development of BE.[25] Van Baal *et al.* also reported that a subset of 23 miRNAs can be used to determine the type of tissue among normal epithelium (NE), BE, and EAC; 16 miRNAs were significantly differently expressed comparing BE with NE, 2 comparing EAC with NE, and 5 comparing EAC with BE.[26] Unfortunately, all experimental results supported by miRNA microarray only, and there were not enough samples to make the results more confirmative. Another report also showed that seven miRNAs (miR-21, miR-143, miR-145, miR-194, miR-203, miR-205, and miR-215), validating by both microarray analyses and real-time reverse transcription-polymerase chain reaction (RT-PCR), were differential expressed among NE, BE, EAC samples. Five miRNAs (miR-21, miR-143, miR-145, miR-194, and miR-215) were significantly unregulated in columnar tissues compared with NE. Of these, expression of miR-143, miR-145, and miR-215 was lower in EAC than in BE, and miR-203 and miR-205 were high in NE and low in columnar epithelia. This particular study suggested that the expression of miRNAs might define disease states in esophageal epithelium, and dysregulation of specific miRNAs could contribute to metaplastic and neoplastic processes in the esophageal mucosa.[27] In Anderson Cancer Center, researchers detected the expression of miRNAs in BE, low-grade intraepithelial neoplasia (LGIN), high-grade intraepithelial neoplasia (HGIN), and EAC, and confirmed that special miRNAs profiles could differentiate the EAC from NSE with almost 100% accuracy.[28] Mathe *et al.* confirmed that the expression levels of miR-21, miR-223, miR-192, and miR-194 were elevated in EAC

patients. Their elegant experiment also showed that the expression levels of miR-375 was elevated in EAC patients but reduced in ESCC patients, and neither miR-21 nor miR-375 is associated with the administration of neoadjuvant chemoradiation therapy.[29] Another study also reported that miR-21 is overexpressed in EAC and BD (BE with dysplasia) as compared with BE or NE by using *in situ* hybridization, suggesting that miR-21 has a role in the progression of BE to EAC.[30] Let7a, miR-200a, and miR-144, as tumor suppressor miRNAs, were significantly downregulated in human BE compared with NE.[31] Fassan and colleagues detected miRNA expression of the progression of BE-LGIN-HGIN-EAC, and consistently disclosed increased expression of six miRNAs and decreased expression of seven others.[32]

MIRNA PROFILES IN ESCC

MiRNAs can also be correlated with different clinicopathological classifications, especially in ESCC. In Japan, Ogawa *et al.* evaluated the expression of miRNAs in 30 primary ESCC and normal paired specimens by qRT-PCR, and found that 22 miRNAs were higher in ESCC than in NE, 4 miRNAs were lower in ESCC than in NE.[33] The miR-34b expression in ESCC was significantly higher than that in the corresponding NE, and it was more highly expressed in tumors with more advanced stages.[34] Guo *et al.*'s microarray analyses identified seven miRNAs that could distinguish malignant lesions from adjacent normal tissues, and some miRNAs could be correlated with the different clinicopathological classifications.[35] Using ESCC tumor samples and noncancerous tissue obtained endoscopically, Inoue and colleagues showed that miR-10a expression was comparably downregulated in the tumors of HGIN and non-invasive ESCC, while the expression levels were elevated in the invasive ESCC tumors. They also detected expression levels of miR-10a in malignant (OE21 and TE10) and non-malignant (Het1A) ESCC cell lines, and found miR-10a was significantly downregulated in ESCC cell lines.[36] Mathe and colleagues also studied miRNAs' expression in ESCC, and revealed that miR-21 was elevated in both noncancerous and cancerous tissue of ESCC patients, and miR-203, which can inhibit proliferation and invasion of ESCC, was reduced in cancerous compared with noncancerous tissue.[29] Another research indicated that 13 miRNAs were significantly differentially expressed among EAC, ESCC, and NE. Of these, miR-194, miR-192, and miR-200c were significantly upregulated in EAC but not in ESCC. Compared with NE, miR-342 is differentially expressed in ESCC but not EAC, and others including miR-21, miR-205, miR-203 and miR-93 are differentially expressed between tumor and normal but not necessarily between the two tumor histologies;[37] however, this study only use miRNA microarray, addition of qRT-PCR could make the results more dependable. Recently, Okumura *et al.* suggested that let-7 and miR-1274 possibly play a role in the expression of p75NTR and/or maintenance of high colony-forming capacity in ESCC.[38] Matsushima and colleagues also found that miR-205 and miR-10a were significantly altered in cellular expression, and might be specific for ESCC, with potential roles in ESCC pathogenesis.[39] Kimura *et al.* suggest that miR-205 might be a specific marker miRNA of both normal and malignant squamous epithelia, and miR-21 might be a putative oncogenic miRNA in ESCC.[40] Wu *et al.* ascertained that a set of miRNAs was deregulated in ESCC tissues, and the expression levels of miR-143 and miR-145 were significantly decreased in most of ESCC tissues examined. Both miR-143 and miR-145 expression correlated with tumor invasion depth.[41]

CIRCULATING MIRNAS IN ESCC

Because serum and plasma are relatively easy to access, circulating biomarkers are one of the most promising means of tumor diagnosis. Previous studies have shown that human serum contains miRNAs, and that the expression pattern of these serum miRNAs can potentially be used to identify various types of cancer.[42–47]

Zhang *et al.* demonstrated marked upregulation of 25 serum miRNAs in ESCC patients compared with controls by Solexa sequencing technology. Their study demonstrated that the 7-miRNA profile may be used as a biomarker for ESCC, and importantly, has the potential to predict ESCC at a relative early stage, as exhibited by the clear separation of stage I/II ESCC samples from the control samples in the cluster analysis dendrogram. Furthermore, they have demonstrated that the panel of seven serum miRNAs is a kind of much more sensitive indicator of ESCC than the conventional carcinoembryonic antigen (CEA) biomarker. Their large-samples experiment showed that serum miRNAs hold promise as a novel blood-based biomarker for the diagnosis of ESCC.[48] Zhang and colleagues certified that serum miR-31 levels in ESCC patients were significantly higher than in normal controls. And patients with high levels of serum miR-31 also had a poorer prognosis in relapse-free and tumor-specific survival. *In vitro* studies also showed that miR-31 promoted ESCC colony formation, migration, and invasion.[49] Komatsu *et al.* also detected circulating miRNAs in plasma of patients with ESCC, and reported that the plasma level of miR-21 was significantly higher but reduced in post-operative samples, patients with a high plasma level

of miR-21 tended to have greater vascular invasion and to show a high correlation with recurrence, and miR-375 was significantly lower in ESCC patients than controls.[50]

MiRNA-RELATED GENES AND ESOPHAGUS TUMORIGENESIS

Ago proteins and trinucleotide repeat containing (TNRC) proteins are main components of the RISC and participate in miRNA-induced gene silencing. The increased expressions of Ago2 and TNRC6A (GW182) in both principal component analysis (PCA) and ESCC compared with their normal cells suggested that overexpression of these proteins may be related to miRNA functions and might play a role in tumorigenesis of ESCC.[51] The expression levels of RNase – one of the miRNA processing enzymes – were elevated in ESCC. Knockdown of RNase in esophageal cancer cell lines resulted in a 46–85% reduction in cell number. In an immunohistochemical study, the intensity of RNase expression was often increased in the tumor compared with that in normal epithelium.[52] In addition, single nucleotide polymorphisms (SNP) in miRNA-related genes may increase the risk of ET. SNPrs6505162, which is located in the pre-mir423 region, was associated with a significantly reduced risk (odds ratio, 0.65; 95% confidence interval, 0.42–0.99), while the polymorphism in the pre-mir196a-2 gene was associated with a 1.7-fold increased the risk of ET, and in the pre-mir631 gene was also associated with a significantly increased ET.[53] Wang and colleagues performed a genetic association study between the SNP (rs11614913) in pre-miRNA-196a and ESCC susceptibility, and found that the homozygote CC of this SNP increased the risk of ESCC compared with the homozygote TT, and the risk was more evident among smokers than non-smokers.[54] SNPrs2910164, which is located in the sequence of the miR-146a precursor, was reportedly not only had a strong correlation with the clinical TNM stage of ESCC, but also had a role to contribute to ESCC susceptibility.[55] Taken together, these exploratory analyses highly suggested that the miRNAs and their related genes might possess physiological significance in the regulation of ET development.

ET develops through progression from NE to LGIN, HGIN, then to cancerization. Intraepithelial neoplasia (IN) is the most predictive marker for risk of ET, and the degree has positive association with ET's onset risk. The following studies in the extent of genome indicated that miRNAs not only had extensive relationship with etiology of ET, especially had much value in risk evaluation of precancerous disease, but also had extensive use in early diagnosis, histological classification, prognostic assessment, and even treatment of ET.

FUNCTIONAL ANALYSIS ON MIRNAS AND TARGET GENES

After 2009, miRNAs' researches mainly focus on target genes and its regulation mechanism in cancer. It is now universally acknowledged that oncogene and tumor-suppressing gene usually have been targets of miRNAs. As a ts-miR, ANXA1 is considered to mediate proliferation and suppress apoptosis. Luthra *et al.* confirmed that miR-196a, which has significant inverse correlation with ANXA1 mRNA levels in ET cell lines, directly targets ANXA1 3'-UTR, thereby exerting antiapoptotic effects and contributing to EAC cell survival.[56] In ESCC, miR-21 has been certified that it can target programmed cell death 4 (PDCD4) at the posttranscriptional level, and regulate cell proliferation and invasion.[57] MiR-106b-25 polycistron, which could affect proliferation, antiapoptosis, and cell cycle promotion in ET cells, may have a role in tumorigenic activity in NE-BE-EAC tissues. It has since been demonstrated that miRs-93 and-106b targeted and inhibited p21 by mRNA degradation, whereas miR-25 targets and inhibits Bim by translational inhibition, resulting in the formation and proliferation of ET.[58,59] LATS2 is a member of the LATS tumor suppressor family, which can regulate the cell cycle and apoptosis. As an oncomiRs, miR-373 participated in esophageal carcinogenesis by directly suppressing LATS2 expression.[60] Another research also ascertained that miR-196a specifically targets the 3'-UTRs of KRT5, SPRR2C, and S100A9 mRNA, reducing the expression of these proteins.[61] Ohashi *et al.* determined the role of miR-200 family members in miRNA-mediated posttranscriptional regulation of ZEB1/2 through Notch signaling, which contributes to ET initiation and progression.[62] Fassan confirmed that miR-21 as a negative regulator of the expression of PDCD4 during multistep Barrett's carcinogenesis.[63] Recently, Matsuzaki *et al.* confirmed that downregulation of p27Kip1, as a consequence of the increased expression of miR-221/222 on the progression of BE to ECA, enhanced the degradation of Cdx2 and cell proliferation during the exposure to bile acid in human esophageal epithelial cells. This founding may play a role in the development of EAC.[64] FSCN1, as tumor suppressor gene, is associated with ESCC carcinogenesis by inhibiting cell growth and invasion. Kano *et al.* first showed miR-145, miR-133a, and miR-133b suppressed cell proliferation and cell invasion in ESCC by inhibiting FSCN1's expression.[65] Ma *et al.* also concluded that MiR-21 could promote the cell proliferation, might target PTEN at posttranscriptional level, and regulated the cancer invasion in Kazakh's ESCC.[66] Let-7, which is lower expressed in ESCC, could inhibit cell proliferation, and there was a correlation between let-7 lower expression and lymph node metastasis in ESCC patients. And

HMGA2 may be negatively regulated by let-7 at the posttranscriptional level in ESCC.[67] Matsushima and colleagues showed that miR-10a, as a tumor suppressor, could control cell migration and invasion by targeting homeobox genes, and alteration of miR-205 expression could modulate the phenotype of epithelial cells toward epithelial-mesenchymal transition (EMT), characterized by reduced abundance of E-cadherin, which is the ESCC-specific miRNA target, and inhibition of the E-cadherin repressors, ZEB1 and ZEB2.[39] However, their latest study demonstrated that miR-205 was an ESCC-specific miR that exerts tumor-suppressive activities with EMT inhibition by targeting ZEB2.[68] KLF4, another known tumor suppressor gene, which has been reported to suppress ESCC cell migration and invasion, was a direct target of miR-10b.[69] Chen and colleagues further demonstrated that miR-92a directly targeted the CDH1 3'-UTR and repressed the expression of CDH1, a tumor metastasis suppressor, and they demonstrated that miR-92a promotes ESCC cell migration and invasion at least partially via suppression of CDH1 expression.[70] MiR-29c was frequently downregulated in ESCC tissues and cells, and suppressed tumor growth by inducing cell cycle G1/G0 arrest mainly through modulating cyclin E expression.[71] MiR-21 is a known oncomiR. A miR-21 target, SOX2, is important in normal esophageal development.[72] Another research showed that transient transfection of miR-34a inhibited c-Met and cyclin D1 expression and ET cell proliferation, whereas miR-16-2 suppressed RAR-(beta) 2 expression and increased tumor cell proliferation.[73] Ito *et al.* revealed that the expression level of miR-593 was inversely correlated with PLK1 mRNA in 48 surgically resected ET tissues. Then, they further demonstrated that PLK1 is posttranscriptionally regulated by miR-593.[74] Li and colleagues revealed that ARTN, a known tumor metastasis-related gene, is a direct target of miR-223 and that miR-223 may have a tumor suppressor function in ESCC.[75] Liu *et al.* concluded that the miR-17-92 cluster is overexpressed in ESCC and that TNF-α is a novel target of miR-19a.[76] MiR-375 was significantly reduced in ESCC tissues compared with NE. Gene expression data and luciferase reporter assays have demonstrated that AEG-1/MTDH was directly regulated by miR-375.[22] Imanaka *et al.* found that miR-141 directly targeted the 3'UTR of YAP1, which is known to have a crucial role in apoptosis induced by DNA-damaging agents, and thus downregulated YAP1 expression.[77] MiR-210, which was downregulated in human ESCC and ESCC cell lines, could inhibit cancer cell survival and proliferation by inducing cell death and cell cycle arrest in G1/G0 and G2/M, and FGFRL1, as a target of miR-210, could accelerate cancer cell proliferation by preventing cell cycle arrest in G 1/G0 in ESCC.[78] MiR-145 via targeting GATA6, transcription factor of bone morphogenetic protein 4, and related signal transduction pathway might play an important role in the development of BE.[79] Recently, miR-203 has been reported to inhibit cell proliferation by repressing DeltaNp63 expression in human ESCC.[80] Alterations in miRNA expression have been shown to not only affect tumor growth but also response to chemotherapy. MiR-148a sensitized sensitive and resistant cells to cisplatin treatment, and had a similar effect on treatment with 5-FU. Downregulation of Pregnane X receptor (PXR), which had been identified as target of miR148a, could explain these findings as PXR upregulates several drug metabolizing enzymes or efflux transporters including CYP3A4, multidrug resistance gene 1 (MDR1), MRP3, or P-glycoprotein (P-gp).[81] Hamano and colleagues indicated that the involvement of miR-200c expression in chemoresistance occurs by directly targeting PPP2R1B following upregulation of Akt signaling in ESCC.[82] We generalized the main kinds of miRNA target genes and their potential roles in ET (Table 1).

In recent 3 years, mounting evidence exists that there was a many-to-many relationship between miRNAs and mRNAs because a single miRNA targets multiple mRNAs, and a single mRNA is targeted by multiple miRNAs.[84,85] As we all know, complex diseases are affected by several miRNAs rather than a single miRNA. Xu *et al.* analyzed target gene of all miRNAs of complex disease (e.g. ovarian cancer, type 2 diabetes), then built the miRNA–miRNA functional synergistic network;[86] they explored miRNA functions at a system-wide level, and made a better understanding about the role of miRNAs in complex diseases.

MIRNAS AND ITS CLINICAL INSIGHTS

MiRNA and early diagnosis

Most ET has been diagnosed in advanced stages, delaying timely treatment and leading to poor outcomes. Therefore, seeking specific molecular changes that could identify patients in early stage of ET will have profound significance. Multiple studies have documented that different diseases of esophagus have different expression profiles of miRNAs, and using different expression profiles of miRNAs can differentiate abnormal tissue from normal tissue with high sensitivity and specificity.[24,27,87] Maru *et al.* found that miR-196a levels were 10- to 100-fold higher in precancerous lesions and EAC than in NE, and then confirmed that the progression of NE-BE-LGIN-HGIN-EAC was associated with a concomitant increase of miR-196a levels, so it may also constitute a good biomarker of progression during BE–EAC carcinogenesis.[61] Interestingly, Fassan also examined the miRNA profiling of NE-BE-LGIN-HGIN-EAC, and disclosed increased expression of six miRNAs

Table 1 MiRNAs and their reported target genes in ET

miRNA	Deregulation	Species	Target	Function	Ref
Let-7	Down	ESCC tissues and cell lines	High mobility group A2 (HMGA2)	Inhibited cell proliferation	67
MiR-10a	Down	ESCC tissues and cell lines	Homeobox genes	Controlled cell migration and invasion	39
MiR-10b	Up	ESCC cell lines	KLF4(Kruppel-like factor 4)	Promoted tumor cell migration and invasion	69
MiR-16-2	Up	ESCC tissues and cell lines	RAR-(beta)2 c-Met,cyclin D1	Increased tumor cell proliferation	73
MiR-19a	Up	ESCC tissues and cell lines	Tumor necrosis factor-(alpha)	Promoted cellular growth	76
MiR-21	Up	BE and EAC tissues and EAC cell line	SOX2 (sex determining region Y-box 2)	Promoted pathogenesis of BE and EAC	72
	Up	ESCC tissues and cell lines	PTEN(phosphatase and tensin homolog)	Promoted tumor cell proliferation	66
	Up	BE-BAC tissues, nude mice	PDCD4(Programmed cell death 4)	Promoted Barrett's carcinogenesis	63
	Up	ESCC tissues and cell lines	PDCD4	Promoted tumor cell proliferation and invasion	57
MiR-25	Up	BE and EAC tissues and EAC cell line	Bim(Bcl2l11)	Promoted neoplastic progression and proliferation	58,59
MiR-29c	Up	ESCC tissues and cell lines	Cyclin E	Induced cell cycle G1/G0 arrest	71
MiR-34a	Up	ESCC tissues and cell lines	RAR-(beta)2 c-Met,cyclin D1	Increased tumor cell proliferation	73
MiR-92a	Up	ESCC tissues and cell lines	CDH1 (cadherin 1)	Promoted tumor cell migration and invasion	70
MiR-93 MiR-106b	Up	BE and EAC tissues and EAC cell line	P21(cyclin-dependent kinase inhibitor 1A)	Promoted neoplastic progression and proliferation	58,59
MiR-133a MiR-133b	Down Down	ESCC tissues and cell lines	FSCN1 (actin-binding protein, Fascin homolog 1)	Cell growth and invasion inhibition	65
MiR-141	Up	ESCC cell lines	YAP1	Resistance to cisplatin-induced apoptosis	77
MiR-145	Down	ESCC tissues and cell lines	FSCN1 (actin-binding protein, Fascin homolog 1)	Cell growth and invasion inhibition	65
	Down	normal esophagus epithelial cell line	GATA6†	Promoted pathogenesis of BE	79
MiR-148a	Up	ESCC tissues and cell lines	Pregnane X receptor (PXR)	Sensitized sensitive and resistant cells to cisplatin and 5-FU treatment	81,83
MiR-196a	Up	EAC tissues	KRT5(keratin 5), SPRR2C(small proline-rich protein 2C),S100A9(S100 calcium binding protein A9)	Promoted BE to EAC	61
	Up	EAC tissues and cell line	Annexin A1 (ANXA1)	Promoted cell proliferation, anchorage-independent growth and suppressed apoptosis	56
MiR-200	Up	ESCC cell lines, nude mice	ZEB1/2‡ (zinc finger E-box binding homeobox 1/2)	EMT inhibition	62
MiR-200c	Up	ESCC tissues, cisplatin-resistant cell	PPP2R1B	Induced chemoresistance of cisplatin	82
MiR-203	Down	ESCC cell lines	(Delta)Np63	Inhibited tumor cell proliferation	80
MiR-205	Up	ESCC tissues and cell lines	ZEB2(zinc finger E-box binding homeobox 2)	EMT inhibition	39,68
MiR-210	Down	ESCC tissues and cell lines	Fibroblast growth factor receptor-like 1 (FGFRL1)	Inhibited tumor cell survival and proliferation	78
MiR-221/223	Up	normal squamous esophagus epithelial cell line	p27Kip1	Enhanced degradation of caudal-related homeobox 2 (Cdx2), promotes BE to EAC	64
MiR-223	Down	ESCC tissues and cell lines	Artemin (ARTN)	Decreased cell migration and invasion	75
MiR-373	Up	ESCC tissues and cell lines	LATS2 (large tumor suppressor, homolog 2)	Stimulated proliferation	60
MiR-375	Down	ESCC tissues and cell lines	AEG-1/MTDH	Increased tumor cell proliferation	22
MiR-593	Down	ESCC tissues and cell lines	Polo-like kinase 1 (PLK1)	Reduced cell proliferation, increased cell proportion of G2/M phase	74

†GATA6 is a transcription factor of bone morphogenetic protein 4 (BMP4). ‡microRNA-mediated posttranscriptional regulation of ZEB1/2 in the presence of DN-MAML. BE, Barrett's esophageal; EAC, esophageal adenocarcinoma; EMT, epithelial-mesenchymal transition; ESCC, esophageal squamous cell carcinoma; ET, esophageal tumor.

Table 2 Potential clinical significance of MiRNA in esophageal diseases

MiRNA	Deregulation	Pathological type	Potential clinical significance	Ref
Early diagnosis				
Let-7c	Up	BE-EAC	Biomarker of early diagnostic tool in EAC	23
MiR-192, miR-215	Up	BE-EAC	Biomarker of early diagnostic tool in EAC	23
MiR-196a	Up	BE-EAC	Biomarker of BE–EAC tumorigenesis	61
MiR-203, miR-205	Down	BE-EAC	Biomarker of BE–EAC tumorigenesis	23,37
MiR-203, miR-205	Down	ESCC	Novel diagnostic tool in ESCC	37
Prognosis judgment				
MiR-16-2	Up	EAC	Lymph node metastasis, shorter overall and disease-free survival	73
MiR-21	Up	ESCC	Spread to distant lymph nodes	83
	Up	ESCC	Lymph node metastasis, venous invasion	88
MiR21, miR205	Up	ESCC	Lymph node positivity	89
MiR-30e, miR-200a	Up	EAC	Shorter overall and disease-free survival	73
MiR-92a	Up	ESCC	Lymph node metastasis, poor prognosis	70
MiR-99b, miR-199a-3p, miR-5p	Up	EAC	Lymph node metastasis	90
MiR-103/107	Up	ESCC	Poor survival	35
MiR-106a, miR-148a	Up	ESCC	Disease recurrence, tumor-related mortality	83
MiR-126	Up	EAC	Negatively associated with tumor differentiation, lymph node metastasis	73
MiR-129	Up	ESCC	Slower survival rate of surgically treated patient	91
MiR143, miR145	Up	ESCC	Recurrence of metastasis	89
MiR-148a	Up	EAC	Negatively associated with tumor differentiation	83
MiR-195p	Up	EAC	Higher pathologic tumor stage	73
MiR-296	Down	ESCC	Long-term survival inhibit cell growth	92
MiR328	Down	ESCC	Invaded deeper	90,93
MiR-375	Down	EAC with BE	Worse prognosis	29
Cancer management				
MiR-21	Up	ESCC	Biomarkers to predict response to DCF regimen†	94
	Up	ESCC cell line	Cellular proliferation and invasion, decreased sensitivity to anticancer drugs	57
MiR-26a	Up	ESCC	Biomarkers to predict response to DCF regimen†	94
MiR-27a	Down	ESCC cell line	Confer sensitivity of anticancer drugs	95
MiR-148a	Up	ESCC cell line	Increased sensitivity to cisplatin	81
MiR-192	Down	EAC	Marker for response in the multimodality therapy	96
MiR-296	Down	ESCC	Confer sensitivity of anticancer drugs	92

†DCF regimen: docetaxel /5-FU/cisplatin combination therapy. BE, Barrett's esophageal; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma.

(miR-215, miR-560, miR-615-3p, miR-192, miR-326, miR-147) and decreased expression of seven others (miR-100, miR-23a, miR-605, miR-99a, miR-205, let-7c, miR-203). And five miRNAs (let-7c, miR-192, miR-203, miR-205, miR-215) were further validated by qRT-PCR, known as suitable markers of cancer progression in other models of epithelial oncogenesis. The achieved results confirmed that specific miRNAs were involved in BE carcinogenesis and that they may represent a novel early diagnostic tool in EAC.[23] Feber *et al.* used PCA to identify the expression of miRNAs, which was able to accurately classify the four main types (NE, EAC, ESCC, and BE), and miR-203 and miR-205 were expressed 2- to 10-fold lower in ESCC and EAC than in NE. The miR-21 expression was 3- to 5-fold higher in both tumors than in NE.[37] This research has demonstrated feasibility of miRNAs to distinguish premalignant cases with ET patients with reasonable clinical accuracy; however, the specific miRNAs need to be evaluated further in multicenter and large sample trials. It is generally accepted that ET develops through progression from IN, as a premalignant condition: the IN of esophagus is the most predictive marker for the risk of EAC, and the degree of IN has positive association with risk of EAC. Unfortunately, researches on the relationship between squamous IN and ESCC are rare. Follow-up research in this field will be expected to discriminate the subtype of patients with squamous IN who might be at high risk of progression to ESCC. We generalized the main dysregulated miRNAs in premalignant disease and early stage of ET (Table 2).

MiRNA and prognosis judgment

Transcriptional profiling of miRNAs expression in resected ET tissues may have clinical utility as predictors of prognosis. Chen *et al.* demonstrated that patients with upregulated miR-92a are prone to lymph node metastasis, and thus have poor prognosis.[70] Akagi and colleagues found that the high expression of mature miR21 and miR205 was associated with lymph node positivity in ESCC patients ($P < 0.05$). The high levels of expression of mature miR143 and miR145 were associated with recurrence of metastasis in ESCC patients ($P < 0.05$). The findings may imply that miRNA biogenesis is

aberrantly accelerated in ESCC.[89] High expression of miR-103/107 also correlated with poor survival by univariate analysis as well as by multivariate analysis.[35] And the reduced levels of miR-375 in cancerous tissue of EAC patients with BE were strongly associated with worse prognosis.[29] Besides, ESCC with low GNG7 expression invaded deeper than those with high GNG7 expression. GNG7 expression was significantly associated with the presence of miR328 in ESCC cell lines, which suggests that miR328 may be a regulator of GNG7 expression, and GNG7 suppression represents a new prognostic indicator in cases of ESCC.[93] Recently, Feber and colleagues indicated that five miRNAs were associated with patient survival independent of node involvement and overall stage. And the expression of three miRNAs (miR-99b, miR-199a-3p, and miR-5p) was also associated with the presence of lymph node metastasis.[90] Downregulation of miR-296 might inhibit growth of ET cells *in vitro* and *in vivo* through regulation of cyclin D1 and p27, confer sensitivity of both P-gp-related and P-gp-nonrelated drugs on ET cells, promote ADR-induced apoptosis accompanied by increased accumulation and decreased releasing amount of ADR, and significantly decrease the expression of P-gp, Bcl-2, and the transcription of MDR1, but upregulate the expression of Bax. Furthermore, low expression of miR-296 was able to distinguish long-term survivors with node-positive disease from those dying within 20 months by predicting survival (median, 23.7 vs. 12.9 months).[92] In Ogawa's work the Kaplan–Meier survival curves showed that the high expression levels of six miRNAs correlated with significantly lower patient survival rate; meantime, the overexpression of miR-129 was identified as a significant and independent prognostic factor ($P = 0.031$) in surgically treated ESCC patients.[33] Nguyen *et al*. showed that transcriptional profiling of inflammation-associated genes and miRNAs expression in resected esophageal Barrett's-associated adenocarcinoma tissues may have clinical utility as predictors of prognosis.[91] RNase – one of the miRNA processing enzyme – was elevated in ESCC. And its expression correlated with poor ESCC prognosis. The prognostic effect of RNase ($P = 0.0036$) seems to be independent of disease stage ($P = 0.0060$).[52] Hummel *et al*. confirmed that alterations in the expression of miR-21 correlate with tumor location and lymph node status, the expression of miR-106a and miR-148a correlates with disease recurrence and tumor-related mortality, miRNAs might inform the initial assessment of ET patients, and predict those at higher risk of post-surgical recurrence.[83] Furthermore, miR-126 expression was associated with tumor cell dedifferentiation and lymph node metastasis, miR-16-2 was associated with lymph node metastasis, and miR-195p was associated with higher pathologic disease stages in patients with EAC. Kaplan–Meier analysis showed that miR-16-2 expression and miR-30e expression were associated with shorter overall and disease-free survival in all ET patients. In addition, miR-16-2, miR-30e, and miR-200a expression were associated with shorter overall and disease-free survival in patients with EAC; however, miR-16-2, miR-30e, and miR-200a expression were not associated with overall or disease-free survival in ESCC patients.[73] In a word, analysis of the expression profiles of miRNAs may provide useful information for evaluation of the staging, prognosis, and treatment of ET patients. We also generalized the main dysregulated miRNAs in prognosis judgment of ET (Table 2).

MiRNA and cancer therapy

Over the past year, there have been many experimental studies between miRNAs and ET management. The lack of effective means of early diagnosis, which leads to patients diagnosed in advanced period, is the main barrier to improve 5-year survival rate of ET. MiRNAs are excellent candidates for novel molecular targeting treatments because of their ability to regulate multiple genes in molecular pathways. Vallbohmer *et al*. found that three miRNAs (miR-192, miR-194, and miR-622) was significantly reduced during neoadjuvant therapy, showing lower levels in post-therapeutic tumor samples, by using an expression cut-off value of 0.63; the sensitivity, specificity, and accuracy of pre-therapeutic miR-192 for assessment of histopathological response was 96%, 82%, and 88%, respectively. Their data support the role of miR-192 as a predictive marker for therapy response in the multimodality therapy of patients with locally advanced ET.[96] MiR-148a transfection resulted in a significantly increased sensitivity to cisplatin treatment in cisplatin-sensitive and resistant cells ($P < 0.014$). There was a trend toward a better response to 5-FU in 5-FU sensitive and resistant cells following miR-148a transfection.[81] Kurashige *et al*. identified that the expressions of miR-21 and miR-26a in the pretreatment biopsy specimens can be biomarkers to predict response to DCF regimen (docetaxel /5-FU/ cisplatin) in patients with ESCC.[94]

As one of the most commonly deregulated miRNAs in many cancers, miR-21 is also significantly associated with pathological stage in ESCC. Patients with lymph node metastasis or venous invasion showed significantly high expression of miR-21 in ESCC cell lines.[88] Inhibition of miR-21 showed significant reduction in cellular proliferation and invasion, and increased sensitivity to anticancer drugs in cancer cell lines. This elegant work might support that chemotherapy combined with miRNA-21 suppression could be more effective than chemotherapy alone.[57] Downregulation of miR-27a could confer sensitivity of both P-gp-related and P-gp-nonrelated drugs on

ET cells, thereby promoting ADR-induced apoptosis, accompanied by increased accumulation and decreased release of ADR. This would significantly decrease the expression of P-gp, Bcl-2, and upregulate the expression of Bax, conferring drug-induced apoptosis by enhancing the Bcl-2/Bax ratio in ET cells, and decreasing the transcription of the MDR1, effectively reversing drug resistance of ET cells.[95] And miR-296 has been demonstrated that has the same function with miR-27a. So, these two might play important roles in the pathogenesis of ET and be considered as a potential target for intervention of the drug resistance.[92] We generalized that the main dysregulated miRNAs have potential clinical value on the management of ET (Table 2).

CONCLUSION

At present, the cause of ET is not yet entirely clear, and tumorigenesis might be associated with genetic background and environmental factors, such as genetic factors, smoking, drinking, diet habit, chronic esophageal injury, etc. The gold standard for surveillance of patients with IN is still endoscopic and biopsy pathology; however, sampling errors in biopsies and differences in histological interpretation limit its use. A lot of documents indicated that miRNAs is a kind of hopeful tumor marker, providing a new tool for clinical early diagnosis, prognosis judgment, and management of ET.

When exploring the mechanism of miRNAs, it is commonly acknowledged that miRNA binding with the 3'UTR of its target mRNA, and inducing mRNA degradation. Besides, miRNAs can also regulate gene expression at the transcriptional level by binding directly to the DNA.[97,98] In conclusion, these data show the complexity and widespread regulation of gene expression by miRNAs, which should be taken into consideration when developing miRNA-based gene therapy.

At present, the rules that govern miRNAs binding and gene silencing are not completely understood. It is still a challenge to clarify miRNA-controlling gene and miRNA-regulated target genes. Many sophisticated studies have been done on miRNA-regulated target genes but little about regulation mechanism of miRNA itself. It is also very important to go on revealing more function and regulation mechanism about miRNAs in this fast-moving field.

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