INVITED REVIEW

MicroRNA provides insight into understanding esophageal cancer
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Keywords
esophageal cancer; microRNA; biomarker.

Abstract
Esophageal cancer remains a leading cause of cancer death worldwide with a distinct geographical distribution. Although the molecular genetics and evolution of this disease have been widely studied over past years, the molecular mechanism of esophageal carcinogenesis is still not well understood. MicroRNAs are now being implicated as playing an important role in the multi-stage progression of tumorigenesis. A number of recent studies have reported that microRNAs shed new light on the pathology of esophageal cancer and herald the potential for exploring novel biomarkers for both diagnosis and treatment of this disease. In this review, we focus on the role of microRNAs in esophageal cancer, as both oncogenes and tumor suppressors, and their clinical potency as biomarkers for diagnosis, treatment and prognosis.

Introduction
Esophageal cancer (EC) is the eighth most common cancer and the sixth most common cause of cancer death worldwide, affecting more men than women.\(^1\) According to the latest estimate by the American Cancer Society, in 2010 approximately 16,640 adults (13,130 men and 3,510 women) would be diagnosed with esophageal cancer and approximately 14,500 deaths (11,650 men and 2,850 women) from this disease would occur in the United States alone.\(^2\) EC consists of two subtypes, squamous cell carcinoma (ESCC) and adenocarcinoma (EAC), in accordance with pathological classification. EC has a distinct geographic incidence; ESCC is the main subtype in East Asia, while EAC is more common in Western countries. The prognosis for EC patients depends on the stage at the time of diagnosis. The 5-year survival rate for EC caught in the early stages is greater than 90 percent, but it drops to less than 15 percent in the advanced and metastatic disease states.\(^3\) It is notable that almost 75% of surgically treated patients have proximal lymph node metastases,\(^4\) suggesting there is an exclusive need for solid and accurate biomarkers for EC for the purpose of prevention and early diagnosis. Although the molecular genetics and evolution of this disease have been widely studied, the pathogenesis mechanism of esophageal carcinogenesis is not completely understood. A better understanding of the machinery of the neoplastic progression and identification of valuable biomarkers for this disease would therefore be highly appreciated.

MicroRNAs (miRNA) are a set of small (average 22 nucleotides in length) single-stranded non-coding RNA molecules, which can suppress the translation or induce the cleavage of mRNA molecules by recognizing and binding its 3′-untranslated region (UTR).\(^4\) A primary miRNA (pri-miRNA) is the transcriptional product of a miRNA gene by RNA polymerase II (Pol II). When binding with the microprocessor complex consisting of the RNase III-type endonuclease, Drosha, and its cofactor, Pasha (DGCR8) in the nucleus, the pri-miRNA can be cleaved into the precursor miRNA (pre-miRNA) with approximately 70 nucleotides in length. Binding with the RNA-induced silencing complex (RISC), a mature miRNA can be guided to its cognate mRNA target for subsequent silencing. To date, approximately 1000 different microRNAs have been identified in human tissues.
miRNA have been found in humans. Most miRNA are evolutionarily conserved and some of them play key roles in human development. Over 30% of mammalian genes are believed to be regulated by miRNAs. Due to their critical functions in gene regulation and expression, miRNAs are reported to involve tumor cell transformation, proliferation, and apoptosis. Following a pivotal study that established a link between B cell chronic lymphocytic leukemia (CLL) and deregulated expression of miR-15a and miR-16-1, miRNA is being recognized as a key player in the pathogenesis of human cancers. It has been demonstrated that more than 50% of miRNA genes are located in cancer-associated genomic regions or within fragile sites, and numerous miRNA have been identified as tumor-related and can be categorized as either oncogene or tumor suppressor depending on their functional relevance. Here, we will focus on the role of miRNA in esophageal cancer and their clinical potency as biomarkers for diagnosis, treatment, and prognosis.

### MiRNAs in EC

Compared to other solid tumors, miRNA and EC have not been intensively studied. To date, there are only 47 research and four review publications archived in PUBMED, and all of them were published after 2008, which demonstrates that the importance of miRNA in EC is being noticed. In these studies, the aberrant expression of several miRNAs such as miR-21 has been repeatedly identified in EC; however, the roles that specific miRNAs play in the progression of esophageal carcinogenesis have yet to be completely tested.

### Oncogenic miRNA in EC

The role of miRNA in tumorogenesis depends on their target genes' classification and abundance. When targeting tumor suppressor genes, these over-expressed miRNA will play a promoting tumor role as oncogenes; likewise, when targeting oncogenes, these miRNA will have the characteristics of tumor suppressors. The first characterized oncogenic miRNA in EC was miR-196a by Luthra et al., who indentified the inverse correlation of this miRNA and the suppression of ANXA1 in both EC cell lines and the paired normal/tumor tissue from 10 EC patients. ANXA1 is a mediator of apoptosis and an inhibitor of cell proliferation documented in many human cancers. Using the luciferase reporter assay, miR-196a was verified to directly repress the expression of ANXA1 through targeting its 3'-UTR, which might interpret the molecular mechanism of miR-196a's oncogenic role in EC promoting cell proliferation and suppressing apoptosis. Using esophageal cultured cell lines and tissues, Kan et al. found that a polycistron consisting of three miRNA, miR-106b, -93, and -25, exerted potential proliferative, anti-apoptotic, cell cycle-promoting affects in vitro and tumorigenic activity in vivo. MiR-93 and -106b can cleave p21 mRNAs and miR-25 can suppress Bim expression at a translational level, which mainly accounts for their oncogenic effects on EC. Lee et al. found that miR-373 was over-expressed in ECSS patient samples and was capable of down-regulating large tumor suppressor homolog 2 (LATS2) at a post-transcriptional level. LATS2 is a member of the LATS tumor suppressor family and is implicated in the regulation of cell growth and cell death. MiR-373 expressed inversely with LATS2 in esophageal cancer cell lines and was believed to stimulate esophageal cancer cell proliferation through inhibiting LATS2. Using serum samples from 290 ESCC patients and 140 age/gender equivalent controls, Zhang et al. discovered 25 elevated serum miRNA relevant to ESCC by Solexa (Illumina, San Diego, CA, USA) sequencing and validated seven of them (miR-10a, -22, -100, -148b, -223, -133a, and -127-3p) with the real-time PCR. However, miR-10a was suggested to act as a tumor suppressor specific to ESCC by controlling cell migration/invasion in another study. This group also proposed that miR-205 was decreased in ESCC cells and modulated the epithelial-mesenchymal transition (EMT) by targeting zinc finger E-box binding homeobox family ZEB1 and ZEB2 resulting in downregulation of E-cadherin.

MiR-21, a well-known oncogenic miRNA in various malignancies, is also the most frequently reported miRNA in EC. Hu et al. reported that miR-21, together with let-7g and miR-195p, were overexpressed in 10 EC cell lines. Kimura et al. found miR-21, -16, and -30a-5p had been upregulated in squamous cell carcinoma of human head and neck (HNSCC) and esophagus (ESCC). A very recent study by Akagi et al. reported similar results, that mature miR-21 expression significantly increased in ESCC compared to normal epithelium, and the ratio of mature to precursor miR-21 was also higher in ESCC than normal epithelium, which implied that mature miR-21 might play an important role in the progression of ESCC. Ma et al. demonstrated that in EC cell line Eca109, when transfected with miR-21 mimics, the expression of tensin homolog deleted from chromosome-10 (PTEN) was decreased to ~40%, resulting in the promotion of cell proliferation; likewise, when miR-21 was suppressed by its inhibitor, PETN increased ~79% in parallel with slowing cell growth. In 18 paired ESCC and adjacent normal tissue samples, miR-21 and PETN exhibited significantly inverse expression. Hiyoshi et al. investigated the expression of miR-21 in EC by comparing matched normal esophageal epitheliums and ESCC and found 18 of 20 cancer tissues overexpressed miR-21 in comparison to their matched normal epithelium. Specifically, tissues taken from patients with lymph node metastases or venous invasion showed significantly higher expression of miR-21. Additionally, the expression of miR-21 was elevated in seven different EC cell lines. They also showed that anti-miR-21-transfected cells had a
significant reduction in cellular proliferation and invasion, which might account for targeting programmed cell death protein 4 (PDCD4) by miR-21 at the post-transcriptional level. Fassan et al. drew the same conclusion by proving that miR-21 negatively regulates the expression of PDCD4 in the setting of Barrett’s carcinogenesis.

Tumor suppressor miRNA in EC

In consideration of the unique signature of miRNA in suppression of cognate genes’ expression at post-transcriptional and translational levels, targeting characterized oncogenes will lead to a tumor suppressing effect of miRNA on human cancer. MiR-145, -133a, and -133b were the first identified tumor suppressing miRNA targeting actin-binding protein fascin homolog 1 (FSCN1) in ESCC. These three miRNA were capable of inhibiting cell proliferation and cell invasion in ESCC cells via repressing FSCN1, an oncogene associated with the carcinogenesis of ESCC. Employing real-time PCR technology, Wu et al. examined miR-143 and -145 expressions in 86 ESCC patient samples and confirmed the down-regulation of both miRNA in ESCC. They also proved that elevated miR-143 and -145 were able to suppress cell mobility in ESCC cells but FSCN1 seemed not involved in this process. Polo-like kinase 1 (PLK1) presents oncogenic features in many human cancers, but its biological function in EC remains unknown. Ito et al. found PLK1 was expressed in 11 EC cell lines but not in normal esophageal epithelial cells (HEEpiC). Knock-down of PLK1 in EC cells induce G2/M arrest, reduce cell proliferation, and decrease tumor formation ability in vivo. Using 48 EC patient samples, they found miR-593* was inversely expressed to PLK1. Overexpressing miR-593* was able to significantly repress the expression of PLK1 leading to reduced cell proliferation and increased cell proportion of G2/M phase in EC cells. Another study by Tsuchiya et al. reported that miR-210 was downregulated in ESCC patient samples and derived cell lines. In in vitro cell models, miR-210 was found to be capable of inducing cell death and cell cycle arrest in G1/G0 and G2/M. Fibroblast growth factor receptor-like 1 (FGFRL1) was identified to accelerate cancer cell proliferation by preventing cell cycle arrest in G1/G0 in ESCC. The authors demonstrated that miR-210 could target FGFRL1 and act as a tumor suppressor to regulate cancer cell proliferation in ESCC cells. Hu et al. reported miR-9 and -20a were not expressed in 10 selective EC cells but additional studies should be implemented to further verify their tumor suppressing roles in EC.

Clinical applications of miRNA in esophageal cancer

A few years ago, we and other groups independently demonstrated that miRNAs were relatively more stable and tolerate RNases better than mRNAs, both in archived tissue samples and in blood samples, which suggests that miRNA have the potential to be valuable, practical and reliable biomarkers for diseases. As a result, miRNA has been intensively studied on its characteristics in clinical applications, such as predicting tumor progression, metastasis, survival, and chemotherapy response.

MiRNA and tumor progression

Barrett’s esophagus (BE) is characterized by a metaplastic transition whereby a columnar-lined epithelium with intestinal metaplasia arises within the squamous epithelium of the distal esophagus. It is a recognized precursor of esophageal adenocarcinoma and can progress through varying grades of dysplasia to cancer. However, the molecular mechanisms of its pathogenesis and progression to cancer remain unclear. Several studies have exclusively reported the potential of miRNA in mediating the development of BE and its progression to cancer.

MiR-196a, a previously reported oncogenic miRNA, was evaluated for its role during the progression of BE to low-grade dysplasia, high-grade dysplasia, and EAC, using micro-dissected paraffin-embedded tissues from 11 patients. MiR-196a presented higher expression in BE, dysplastic lesions, and EAC than normal squamous mucosa, whereas miR-196a was more abundant in high-grade dysplasia than in low-grade dysplasia and BE, which implies that the relative quantitation of this miRNA might be associated with the progression of EC. Moreover, using an additional 10 fresh frozen samples, Maru et al. identified that three genes, keratin 5 (KRT5), small proline-rich protein 2C (SPRR2C), and S100 calcium-binding protein A9 (S100A9), that were downregulated during BE progression, could be targeted by miR-196a. This study demonstrated that miR-196a plays an important role in the tumorigenesis and progression of EC. A study performed by Dr Wu’s lab at the MD Anderson Cancer Center, Huston, Texas, identified the miRNA expression signature in BE and EAC. Employing the paired tumor/normal tissue from 16 BE patients with either low- or high-grade dysplasia, or EAC, Dr Wu and her colleagues found BE patients with high-grade dysplasia and EAC had obviously different miRNA profiles compared to normal tissue, but this was not so for BE patients with low-grade dysplasia. Additionally, they validated this result using an additional 75 pairs of tumor/normal tissue samples and drew the conclusion that miRNA signature could indicate the progression of EC. Wijnhoven et al. identified 44 differentiated miRNA using microarray analysis from 16 BE patients with intestinal metaplasia and EAC. They found miR-21, -143, -145, -194, and -215, had higher expression in columnar tissues than in normal squamous epithelium. MiR-143, -145 and -215 were downregulated in EAC when compared to BE. Inversely,
miR-203 and -205 were high in normal squamous epithelia but low in columnar epithelia, and miR-205 levels were lower in gastric epithelium than in both BE and EAC. These results imply that miRNA is able to distinguish the different states of EC and it may contribute to the progression of this disease. Another study led by Fassan et al. used 14 BE patients who contributed a series of sample sets including native stratified squamous epithelium, Barrett’s mucosa, low-grade intraepithelial neoplasia, high-grade intra epithelial neoplasia, and EAC. This study indentified a panel of miRNA consisting of six upregulated (miR-215, -560, -615-3p, -192, -326 and -147) and seven downregulated miRNA (miR-100, -23a, -605, -99a, -195, -let-7c and miR-203) that were tightly correlated with BE progression. Most recently, a phase 2 biomarker study investigated nine miRNA (miR-15b, -21, -192, -205, -486-5p, -584, -1246, -let-7a, and -7d) in 22 BE patients equally with and without dysplasia. Their results reported that the sensitivity and specificity of miRNA for predicting high grade dysplasia or EAC were: miR-15b, 87 and 80%; miR-21, 93 and 70%; miR-203, 87 and 90%; miR-486-5p, 82 and 55%; and miR-let-7a, 88 and 70%.

**MiRNA and EC prognosis**

The first study with a clinical focus on miRNA in EC by Guo et al. investigated the expression of miRNA in EC tissues (31 pairs of tumor/normal tissue samples for the training set and 24 pairs of samples for the validation set) using microarray techniques, in which they found that five miRNA (miR-335, -181d, -25, -7 and -495) could define the gross pathological classification (fungating versus medullary) and two miRNA (miR-25 and 130b) were correlated with tumor differentiation (high versus middle versus low). High expression of miR-103 and -107 were associated with poor survival. These results imply that miRNA expression profiles are capable of being valuable prognostic markers in EC. In a later study, let-7 expression was measured in EC cell Eca109 and 45 pairs of fresh ESCC and normal adjacent tissues. Let-7 was underexpressed in ESCC tissues when compared to normal controls, and its down-regulation was associated with lymph node metastasis. High mobility group A2 (HMGA2) was one of putative target genes of let-7 and it presented inverse association between miRNA expression and major histological types of EC. As discussed before, miR-21 is a well documented oncogenic miRNA. In EC, its molecular characteristics are also associated with prognosis. Mori et al. examined 38 ESCC specimens and matched non-cancerous mucosa for miR-21 expression and they found miR-21 expression was not only higher in tumors than in the non-cancerous controls, but also was significantly elevated in advanced tumors (T3 or T4) than in T1/T2 tumor (P = 0.0333). Additionally, high miR-21 expression closely correlated with the increased invasive potential in ESCC patients. The predation feature of miR-21 on EC metastases was also confirmed by another group, which proved that miR-21 was significantly upregulated in patients with lymph node metastasis and venous invasion. Akagi et al. also demonstrated that miR-21, combined with miR-205, was associated with lymph node positivity in ESCC patients (P < 0.05). Interestingly, they found that high expression of two tumor suppressing miRNA, miR-143 and -145, was associated with the recurrence of metastasis in ESCC patients (P < 0.05), which is contrary to previous reports and needs to be further investigated.

MiR-10b is characterized by its promoting of tumor cell metastasis and invasion in various human cancers with the capability of directly suppressing a number of genes and signaling pathways involved in metastasis. Recently, Tian et al. analyzed the effect of miR-10b on cell motility and invasive-ness using eight ESCC cell lines and they found the expression level of miR-10 strongly correlated with ESCC cell motility and invasion. Krüppel-like factor 4 (KLF4), a zinc finger protein of the Krüppel-like factor family, defined as an inhibitor of EC cell migration and invasion, was targeted by miR-10b and the repression of KLF4 by miR-10b probably accounted for the mobility of ESCC cells. Another study initiated by Feber et al. profiled the global miRNA in 45 primary EAC patients using Affymetrix microarray (Santa Clara, CA, USA). They found the expression of three miRNA (miR-99b, -199a-3p, and -199a-5p) was associated with the presence of lymph node metastasis. Additionally, the signature combining the expression of miR-143, 145, -100, -199a-3p, and -199a-5p significantly correlated with patient survival (P = 0.005).

Quantifying 73 miRNA in 30 primary ESCC samples using real-time PCR, Ogawa et al. determined that the overexpression of six miRNA (miR-23a, -26a, -27b, -96, -128b, and -129) was strongly correlated with a low patient survival rate computed by log-rank test. Especially, miR-129 was identified as an independent factor for prognosis in ECSS, where the hazard ratio for the prediction of early death was 18.11 for high versus low expression levels of miR-129. Validation run on an additional set consisting of 19 patients confirmed these results.

A multicenter study investigated the global miRNA profiles on 100 EAC and 70 ESCC samples and determined the association between miRNA expression and major histological types of EC. They found miR-21 was upregulated and miR-375 was downregulated in ESCC samples when compared to non-cancerous tissue. MiR-194 and -375 had higher expression in EAC than in ESCC. Expressed miR-21 in noncancerous tissue in ESCC and absent miR-375 in EAC with BE were significantly associated with poor prognosis. In another study, Dr Harris reported that miR-375 could improve the accuracy of predicting prognosis of EC when combined with the inflammatory risk score computed by inflammatory gene expression values. A later study from a Chinese group...
| MicroRNA | Function                        | Expression | Target gene | EAC/ESCC          | Publication                                      |
|----------|--------------------------------|------------|-------------|-------------------|-------------------------------------------------|
| let-7a   | Prognostic Marker              | Up         | n/a         | EAC               | (Bansal et al., 2011)³⁴                          |
| let-7c   | Progression Marker             | Down       | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| let-7g   | Oncogene                       | Up         | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁶                             |
| miR-100  | Progression Marker             | Down       | n/a         | EAC               | (Fassan et al., 2010)³⁷                          |
| miR-106  | Response Marker                | Up         | n/a         | EAC/ESCC          | (Hummel et al., 2011)²⁶                          |
| miR-106b | Oncogene                       | Up         | P21         | EAC               | (Kan et al., 2009)³⁸                             |
| miR-126  | Prognostic Marker              | Up         | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁹                             |
| miR-143  | Progression Marker             | Down       | n/a         | EAC/ESCC          | (Akagi et al., 2011; Feber et al., 2011)²¹,³³    |
| miR-145  | Prognostic Marker              | Down       | n/a         | EAC/ESCC          | (Wijnhoven et al., 2010)³²                        |
| miR-147  | Progression Marker             | Up         | n/a         | EAC               | (Fassan et al., 2010)³⁹                          |
| miR-148  | Response Marker                | Up         | n/a         | EAC/ESCC          | (Hummel et al., 2011)²⁶                          |
| miR-15b  | Prognostic Marker              | Up         | n/a         | EAC               | (Bansal et al., 2011)³⁴                          |
| miR-16   | Prognostic Marker              | Up         | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁶                             |
| miR-192  | Progression Marker             | Up         | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-194  | Prognostic Marker              | Up         | n/a         | EAC               | (Mathe et al., 2009)⁴¹                           |
| miR-195p | Oncogene, Prognostic Marker    | Up         | ANXA1       | EAC/ESCC          | (Hu et al., 2011)³⁹                             |
| miR-196a | Oncogene                       | Up         | KRT5, SPRR2C, S100A9 | EAC        | (Maru et al., 2009)⁵⁰                          |
| miR-199a-3p | Prognostic Marker          | Up         | n/a         | EAC               | (Feber et al., 2011)³⁹                          |
| miR-199a-5p | Prognostic Marker      | Up         | n/a         | EAC               | (Feber et al., 2011)³⁹                          |
| miR-20   | Tumor Suppressor               | Down       | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁶                             |
| miR-200a | Prognostic Marker              | Up         | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁶                             |
| miR-203  | Prognostic Marker              | Up         | n/a         | EAC               | (Bansal et al., 2011)³⁴                          |
| miR-205  | Progression Marker             | Down       | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-21   | Oncogene                       | Up         | n/a         | EAC/ESCC          | (Akagi et al., 2011; Hamano et al., 2011)²¹,³³,³⁹,⁴⁶ |
| miR-215  | Prognostic Marker              | Up         | PDCD4       | EAC/ESCC          | (Fassan et al., 2010; Hiyoshi et al., 2009)²²,²³ |
| miR-23a  | Progression Marker             | Down       | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-25   | Oncogene                       | Up         | Bim         | EAC               | (Kan et al., 2009)³⁶                             |
| miR-30e  | Prognostic Marker              | Up         | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁶                             |
| miR-326  | Progression Marker             | Up         | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-375  | Prognostic Marker              | Down       | n/a         | EAC/ESCC          | (Mathe et al., 2009; Nguyen et al., 2010)⁴¹,⁴²   |
| miR-486-5p | Prognostic Marker          | Up         | n/a         | EAC               | (Bansal et al., 2011)³⁴                          |
| miR-560  | Prognostic Marker              | Up         | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-593* | Tumor Suppressor               | Down       | PLK1        | EAC/ESCC          | (Ito et al., 2010)⁵⁰                            |
| miR-605  | Prognostic Marker              | Down       | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-615-3p | Prognostic Marker          | Up         | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-9    | Tumor Suppressor               | Down       | n/a         | EAC/ESCC          | (Hu et al., 2011)³⁶                             |
| miR-93   | Oncogene                       | Up         | P21         | EAC               | (Kan et al., 2009)³⁶                             |
| miR-99a  | Prognostic Marker              | Down       | n/a         | EAC               | (Fassan et al., 2010)³⁵                          |
| miR-99b  | Prognostic Marker              | Up         | n/a         | EAC               | (Feber et al., 2011)³⁹                          |

*The expression level of the designated miRNA relevant to tumorigenesis, advanced stage, poor prognosis, or chemoresistance. EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; n/a, not applicable.
### Table 2  MicroRNA and esophageal squamous cell carcinoma

| MicroRNA | Function | Expression | Target gene | EAC/ESCC | Publication |
|----------|----------|------------|-------------|----------|-------------|
| let-7g   | Oncogene | Up         | n/a         | EAC/ESCC | (Hu et al., 2011)19 |
| miR-103  | Prognostic Marker | Up         | n/a         | ESCC     | (Guo et al., 2008)3 |
| miR-106  | Response Marker | Up         | n/a         | EAC/ESCC | (Hummel et al., 2011)26 |
| miR-107  | Prognostic Marker | Up         | n/a         | ESCC     | (Guo et al., 2008)3 |
| miR-10a  | Oncogene | Up         | n/a         | ESCC     | (Zhang et al., 2010)16 |
| miR-10b  | Prognostic Marker | Up         | KLF4        | ESCC     | (Tian et al., 2010)38 |
| miR-126  | Prognostic Marker | Up         | n/a         | ESCC     | (Hu et al., 2011)19 |
| miR-127-3p | Oncogene | Up         | n/a         | ESCC     | (Zhang et al., 2010)16 |
| miR-128b | Prognostic Marker | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |
| miR-129  | Prognostic Marker | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |
| miR-130b | Prognostic Marker | Up         | n/a         | ESCC     | (Guo et al., 2008)3 |
| miR-133a | Tumor Suppressor | Down       | FSCN1       | ESCC     | (Kano et al., 2010)24 |
| miR-133b | Tumor Suppressor | Down       | FSCN1       | ESCC     | (Kano et al., 2010)24 |
| miR-143  | Prognostic Marker | Up         | n/a         | EAC/ESCC | (Akagi et al., 2011; Feber et al., 2011)21,39 |
| miR-145  | Prognostic Marker | Up         | n/a         | EAC/ESCC | (Akagi et al., 2011; Feber et al., 2011; Hamano et al., 2011)21,39,46 |
| miR-148  | Response Marker | Up         | n/a         | EAC/ESCC | (Kano et al., 2010; Wu et al., 2011)24,25 |
| miR-181d | Prognostic Marker | Up         | n/a         | ESCC     | (Hu et al., 2011)19 |
| miR-195p | Oncogene, Prognostic Marker | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |
| miR-200a | Tumor Suppressor | Up         | n/a         | EAC/ESCC | (Kano et al., 2010; Wu et al., 2011)24,25 |
| miR-201  | Oncogene | Up         | n/a         | ESCC     | (Zhang et al., 2010)19 |
| miR-205  | Prognostic Marker | Up         | ZEB         | ESCC     | (Akagi et al., 2011; Matsushima et al., 2011)18,21 |
| miR-214  | Prognostic Marker | Up         | n/a         | EAC/ESCC | (Akagi et al., 2011; Hu et al., 2011; Kimura et al., 2010)19,39,46 |
| miR-210  | Tumor Suppressor | Down       | FGFR1       | ESCC     | (Tsuchiya et al., 2011)27 |
| miR-22   | Oncogene | Up         | n/a         | ESCC     | (Zhang et al., 2010)16 |
| miR-23a  | Oncogene | Up         | n/a         | ESCC     | (Zhang et al., 2010)16 |
| miR-25   | Prognostic Marker | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |
| miR-26a  | Prognostic Marker | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |
| miR-27a  | Response Marker | Up         | n/a         | ESCC     | (Zhang et al., 2010)44 |
| miR-27b  | Prognostic Marker | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |
| miR-296  | Response Marker | Up         | Bax         | ESCC     | (Hong et al., 2010)45 |
| miR-30a-5p | Oncogene | Up         | n/a         | ESCC     | (Kimura et al., 2010)20 |
| miR-30e  | Prognostic Marker | Up         | n/a         | EAC/ESCC | (Hu et al., 2011)19 |
| miR-335  | Prognostic Marker | Up         | n/a         | ESCC     | (Guo et al., 2008)3 |
| miR-373  | Oncogene | Up         | LAST2       | ESCC     | (Lee et al., 2009)15 |
| miR-375  | Prognostic Marker | Down       | n/a         | EAC/ESCC | (Mathe et al., 2009; Nguyen et al., 2010)31,42 |
| miR-495  | Prognostic Marker | Up         | n/a         | PDK1     | (Li et al., 2011)21 |
| miR-593* | Tumor Suppressor | Down       | PLK1        | EAC/ESCC | (Ito et al., 2010)47 |
| miR-9    | Tumor Suppressor | Down       | n/a         | EAC/ESCC | (Hu et al., 2011)19 |
| miR-96   | Tumor Suppressor | Up         | n/a         | ESCC     | (Ogawa et al., 2009)40 |

*The expression level of the designated miRNA relevant to tumorigenesis, advanced stage, poor prognosis, or chemoresistance. EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; n/a, not applicable.
showed that the hypermethylation of the miR-375 gene led to its ectopic expression of miR-375 in EC, and epigenetic silencing of miR-375 induced an upregulation of its targets, 3-phosphoinositide-dependent protein kinase-1 (PDK1). They used both EC cell lines and patient samples. Hu et al. reported that miR-126 expression was associated with tumor cell dedifferentiation and lymph node metastasis, and miR-195p was specific to advanced EAC. MiR-16-2 expression could be linked to lymph node metastasis. The combination of miR-16-2, miR-30e and miR-200a were associated with the shortened survival of EAC patients but not of ESCC patients.

**MiRNA and EC chemotherapy**

The unique signature of miRNA includes regulating multiple cognate genes’ expression via translational repression which is also an important checkpoint through which chemotherapy drugs exert anti-cancer activities on tumor cells. Zhang et al. first investigated the role that miRNA plays in the multidrug resistance of EC cells. They found that the down-regulation of miR-27a could enhance sensitivity of EC cells to both P-glycoprotein-related (vincristine and adriamycin) and P-glycoprotein-non-related drugs (cisplatin and 5-fluorouracil (5-FU)-) and also promoted adriamycin-induced apoptosis. These findings suggest that miR-27a may be involved in the multidrug resistance of EC. In another study, Hong et al. showed that the downregulation of miR-296 had very similar effects as miR-27 on multiple drug resistance in EC. They found that miR-296 was able to target cyclin D1 and p27 leading to the inhibition of cancer cell growth, and that downregulation of miR-296 caused the increase of Bax expression, the parallel decrease of growth, and that downregulation of miR-296 caused the inhibition of cancer cell viability after both cisplatin and 5-FU treatments. However, miR-106 had inconsistent effects on EC cells in response to the two drugs by increasing resistance to cisplatin and sensitivity to 5-FU. In a recent study initiated by Hamano et al., a number of miRNA were evaluated for the correlation of their expression and the prognosis or response to chemotherapy in 98 formalin-fixed, paraffin-embedded EC samples. Overexpression of miR-21 and underexpression of miR-145 were associated with a shortened duration of patient survival, which is consistent with their oncogenic and tumor suppressing roles, respectively. MiR-200c was overexpressed in EC samples and not only correlated to the short survival duration, but also associated with the resistance of EC cells to cisplatin. The effect of miR-200c on chemoresistance was believed to be related to target PPP2R1B, a subunit of protein phosphates 2A, resulting in the suppression of Akt signaling transduction.

**Summary**

Based on the knowledge reviewed above, we have a clear picture that miRNA plays an important role, at least in part, in the diverse molecular mechanisms leading to the formation and progression of EC. These studies are from both laboratory and clinical settings, which evidence the perspective of miRNA as one of the most valuable biomarkers and therapeutic targets in the view of current EC research. Tables 1 and 2 summarize most of the miRNA associated with EC and their molecular characteristics. We gladly find the research trend of miRNA and EC has changed from solely searching altered specific miRNA to exploring molecular networks and connections between miRNA and signaling pathways involved in the progression of EC. It is certain that a better understanding of the biological mechanisms of miRNA function will allow us to visibly observe the genetic impacts on carcinogenesis and to explore effective therapeutic strategies to conquer EC in the near future.

**Acknowledgments**

We appreciate the contribution of all colleagues who are working on this topic. Due to the overwhelming accomplishments in this field, we apologize that we could not cite all publications in this review due to space limitations and timing.

**Disclosure**

No authors report any conflict of interest.

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