MicroRNA and its roles in esophageal cancer

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Summary

Esophageal cancer is the eighth most common cancer and causes the sixth highest cancer-related mortality worldwide. The 5-year survival of patients suffering from esophageal cancer in either advanced stage or metastasis is less than 20%. MicroRNAs are small, well conserved, non-coding RNA molecules that either repress translation or promote mRNA degradation based on the degree of complementary between miRNAs and mRNAs. Based on biogenesis and function of microRNAs, specific microRNA profiles, either from cancerous tissues or serum, were able to serve as diagnostic and prognostic biomarkers of esophageal cancer and predicted the effectiveness of surgery and chemoradiotherapy. MicroRNAs could also influence the biological behaviors of esophageal cancer cells, such as cellular proliferation, apoptosis, invasion and metastasis. MicroRNAs were also associated with multi-drug resistance of esophageal cancer. Further studies on the roles of microRNAs in esophageal cancer would provide a strategy to prevent and treat esophageal cancer, and reverse multi-drug resistance of esophageal cancer.

key words: biological behavior • diagnosis • esophageal cancer • microRNA • multi-drug resistance • prognosis

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Biogenesis of MiRNAs

MiRNA genes are located in different genomic locations, such as introns or regions between genes, and assembled in clusters or dispersedly [22–26]. Some of the miRNA genes from introns share the same promoters and regulators of function genes, and, conversely, the miRNAs could coordinate fine-tune expression of function genes [27–30]. Most mammalian miRNA genes are first transcribed into long primary miRNA (pre-miRNA) which are 5' capped and 3' polyadenylated in the nucleus by RNA polymerase III or III [31–33]. The pre-miRNA transcripts comprise 1 or more hairpin structures delineated by a ~32nt long imperfectly base-paired stem, a terminal loop and 2 single-stranded flanking regions upstream and downstream of the hairpin, consequently producing 1 or more functional mature miRNAs by a series of splicing and processing procedures [34–38].

The miRNAs maturing process has been divided into 2 pathways: the canonical pathway and the non-canonical pathway. In the canonical pathway, 2 steps occur to achieve the functional miRNAs: pre-miRNAs are processed into precursor miRNAs (pre-miRNAs) by Drosophila, which is a member of polymerase RNAase III family in complex with Dicer. The pre-miRNAs are processed into mature miRNAs by Dicer, which is another member of the polymerase RNAase III family in complex with Human Immunodeficiency Virus Transactivation-responsive RNA-binding Protein (TRBP) in the cytoplasm. Dicer engages with DGC8 and cofactors such as DEAD Box RNA Helicase p68 and p72, as well as heterogenous nuclear Ribonucleoprotein (hnRNP), assembling the Microprocessor complex [39]. Microprocessor complex is capable of mediating pre-miRNA cleaving by taking away 3' and 5' end arms of hairpin, subsequently producing a ~70nt long pre-miRNA [23,40–42]. Each element of the Microprocessor complex plays a specific role in pre-miRNA processing. DGC8 stretches out 2 dsRNA-binding domains that connect to the junctions between single-stranded and double-stranded regions of the pre-miRNA stem, directing Drosophila to crop ~11bp single strands from pre-miRNA [39,42,43]. Drosophila also is composed of 2 RNAase domains that can cleave 3' and 5' arms of hairpin and produce a 2nt 3' overhang in the stem [36,44,45]. Other cofactors may function as promoting fidelity, specificity and cleavable activity of Drosophila.

Upon nuclear processing, pre-miRNAs are exported from nucleus to cytoplasm by nuclear exporters, particularly Exportin 5 (Exp5). The precise recognition of Exp5 depends on the RanGTP-dependent pathway, minimal-helical structure, 2nt 3' overhang and the defined stem length of pre-miRNAs. Exp5 also could protect pre-miRNAs from nuclear digestion [25,35,46–49]. Once pre-miRNAs reach the cytoplasm, Dicer immediately recognizes and cleaves pre-miRNA hairpins at junctions between stem and loop, producing a ~22nt RNA duplex [50–54]. TRBP, a crucial cofactor of Dicer, has 3 dsRNA-binding domains and maintains stability between pre-miRNA and Dicer. Dicer, in combination with TRBP, constitutes another Microprocessor in cytoplasm [55–59]. Upon being processed by Microprocessor in cytoplasm, a resultant miRNA/miRNA duplex emerges. This short RNA duplex routinely releases 1 strand (the passenger strand), which is consequently graded, while the other strand (the guide strand), with a less stable 5' hydrogen bond, is incorporated into an Argonaute-containing RNA-induced Silencing Complex (RISC) that is able to mediate gene expression silencing [60,61]. But why the guide strand is incorporated into RISC, but not the passenger strand, is a puzzling question. Generally speaking, the intrinsic characteristics of the miRNA duplex may help answer this question [34,55,62]. The paramount determination of strand selection may be the duplex thermodynamic asymmetry. The 5' end is less stably base-paired and more frequently selected as the guide strand, whereas the 3' end is more stably base-paired and is chosen as the passenger strand [63,64]. Several studies also revealed that a portion of miRNA* were not degraded and have functions similar to those of mature miRNAs. This means that both miRNAs and their complementary strands may be functional [65,66].
of genes, and are spliced and debranched by splicing machinery and lariat debranching enzyme, subsequently forming pre-miRNA-like hairpins. The Mirtron pathway merges with the canonical pathway during hairpin export by Exportin-5, and both types of hairpins are subsequently processed by Dicer [67,68].

Likewise, miRNAs may originate from small nucleolar RNAs (snoRNAs) characterized by 2 pre-miRNA-like hairpins bridged by a hinge [69]. snoRNAs are a group of ~70–200 nt-long conserved small RNAs and parts of small nucleolar Ribonucleoprotein (snoRNP) complex functioning as an enzymatic modification of ribosomal RNA (rRNA) [70]. Dicer, not Drosha, mediates the processing from SnoRNA to miRNA. Endogenous short-hairpin RNAs (endo-shRNAs) and tRNA precursors may be the third origin of miRNAs. Endo-shRNAs are also structured by 2 pre-miRNA-like hairpins bridged by a hinge, similar to snoRNAs [70,71]. The first hairpins of endo-shRNAs are released by cropping at the 5’ end of the second hairpins, serving as suitable substrates for Dicer in cytoplasm [69].

**FUNCTION OF miRNAs**

MiRNAs exist in organisms from procaryotes to eukaryotes. In animals, miRNAs imperfectly base-pair with 3’ Untranslated Region (UTR) of target mRNAs, disturbing target genes expression by either translation repression or target mRNAs degradation. Degradation of mRNAs is mediated by miRNAs deadenylation and/or decapping [72]. In plants and a minority of animals, miRNAs perfectly match 3’ UTR of target mRNAs, consequently initiating a sequence-specific cleavage [73]. The specificity and accuracy of matching between miRNAs and miRNAs largely depend on “seed region”, which is defined as a fraction of nucleotides positioned 2–8 from the 5’ end of miRNA. The “seed region” perfectly base-pairs with 3’ UTR, and thus it is important to delineate the repertoire of miRNAs [74,75]. MiRNAs induce target genes silencing via directly interacting with Argonaute (AGO) and Glycine-tryptophan protein of 182kDa (GW182). AGO and GW182 are cores of miRNA-Inducing Silencing Complex (miRISC) [76].

MiRNAs are recognized as negative regulators in the process of target genes expression as evidenced by a series of in vivo and in vitro studies, but the exact mechanisms by which miRNA leads to mRNAs degradation and translation repression are unclear. In order to clarify whether translation repression occurs in initial [77–82] or post-initial stage [83–86], further studies have been performed. Eulalio [87] indicated translation repression might simultaneously occur at initial and post-initial stages, which included peptide chain elongation, premature translation termination and co-translation protein degradation.

Several distinct insights into how miRISC represses translation initiation have been achieved. A large quantity of evidence suggested the functional 7-methylguanine cap and polyadenylic tail are prerequisite for miRNA-mediated translation repression [77,78,88]. Kiriaikidou [79], Mathonnet [80] and Thermann [89] demonstrated that AGO was able to bind 5’ cap structure via competing with eukaryon Initiation Factor 4E (eIF4E), inducing translation repression. AGO contains 2 phenylalanine residues located in the center of the structure, while eIF4G similarly has 2 tryptophan residues. AGO, instead of eIF4G, engages with 5’ cap, inhibiting assembly of mRNA into 40S pre-initiation complex. Behm-Ansmant [90], Giraldes [91] and Wu [92] demonstrated miRISC induced polyadenylic tail deadenylation, shutting off the interaction between cap structure and free-tail, inducing translation repression. It was discovered that GW182 was the key factor for mRNA deadenylation because of interacting with AGO and Polyadenylic Acid-Binding Protein (PABP) [93,94]. It was also found that miRISC interfered with assembly of 60S ribosomal subunit and 40S pre-initiation complex, inducing translation repression. The emergence of eIF6 was responsible for 60S ribosomal subunit maturation, and prevented premature 60S and 40S subunit from assembling. eIF6 could be disturbed by miRISC, repressing translation by preventing 40S and 60S ribosomal subunits from assembling.

Degradation of miRNAs is another result of miRNA-induced target genes silencing. It was shown that decreased levels of miRNAs probably were related to increased miRNAs degradation [90–92,95,96]. Also, mRNA degradation may be ascribed to mRNA deadenylation, decapping and exonucleolytic digestion [90–92]. The determinants of miRNA-mediated genes expression repression were associated with the number, the type and the position of mismatch in miRNA/mRNA duplex [97].

**miRNAs and esophageal cancer**

With promotion of high-throughput profiling platforms (such as miRNA Microarray, MNA) [17,98], differential expression of miRNAs has been identified in normal tissues vs. pathological tissues and in the same tissues at different stages. Aberrant miRNA profiles in cancerous tissues have potential to distinguish different histopathological types in cancerous tissues [99], suggesting aberrant miRNA profiles might serve as a diagnostic tool for tumors. The miRNA profiles from solid tumors and hematological malignancy have been studied extensively, such as lung cancer [100], breast cancer [101], gastric cancer [102], prostate cancer [103], colorectal cancer [104], and chronic lymphocytic leukemia [105]. Mitchell [106] indicated upregulated miR-141 in serum of prostate cancer patients, to some extent, could serve as a diagnostic biomarker. In addition to the diagnostic role, miRNAs also could potentially be used as prognostic factors predicting tumor recurrence. In non-small-cell lung cancer, upregulated miR-155 and downregulated let-7a indicated poor overall prognosis [100]. It was suggested that the specific miRNA profiles were tightly associated with prognosis and histopathological features simultaneously, such as stages and types. Iorio [101] demonstrated that the miRNA profile of breast cancer was closely associated with tumor stages and types, tumor proliferation index, vascular invasion, and receptor status of estrogen and progesterone in breast cancer. We expect to recognize specific biomarkers unique to esophageal cancer, guiding tumors diagnosis and prognosis. Further study of miRNA profiles gives us an opportunity to predict tumor genesis and turnover.

**MiRNAs as diagnostic biomarkers**

MMA, as a high-throughput screening means, is designed to detect and quantify miRNAs using the Applied Biosystems
real-time PCR instrument. This technology is highly quantitative, highly sensitive, high specificity, high speed and convenient. Feber [19] detected the miRNA profiles from 29 tissues including 10 ADC, 10 SCC and 9 normal esophageal epithelia by MMA, revealing that the levels of miR-194, miR-192 and miR-200c from ADC were upregulated, whereas the level of miR-342 from SCC was downregulated. The levels of miR21 and miR95 were upregulated simultaneously in ADC and SCC, and the levels of miR205 and miR93 downregulated. Mathé [107] collected 170 pairs of cancerous tissues and adjacent normal tissues from 100 ADC patients and 70 SCC patients; the levels of miR-21, miR-223, miR-192 and miR-194 were upregulated, while the level of miR-203 was downregulated in ADC. Highly expressed miR-21 and lowly expressed miR-375 were discovered in SCC. Kan [108], using non-immortalized primary normal esophageal epithelia (HEEpiC) and EAC-derived cell line (OE-33), indicated the level of miR-106b-5p polycystron, which was composed of miR-25, miR-93 and miR-106b in OE-33, was higher than that in HEEpiC.

The specific miRNA profiles might serve as efficient classifiers distinguishing different clinicoopathological types of esophageal cancer. Mathé [107] evaluated the predictive capacity of miRNA profiles in esophageal cancer. The predictive rate of ADC was increased to 77% in ADC patients with Barrett’s esophagus (BE); 78% accuracy was achieved when discriminating ADC with BE from ADC without BE. The miRNA profile of SCC also achieved 82% accuracy when comparing cancer with normal squamous epithelia (NSE). Yang [109], collecting the specimens from BE with low-grade dysplasia (LGD), BE with high-grade dysplasia (HGD), ADC and NSE, demonstrated the predictive rates were 60%, 90% and 100% in BE with LGD, HGD and ADC, respectively, vs. NSE. The specific miRNA profiles were closely associated with TMN staging. Ogawa [110] investigated the association of primary tumor (T), lymph node metastasis (N), vascular invasion (VI) and lymphatic invasion (LI) in SCC with the specific miRNA profiles, and found T factor was correlated with 12 miRNAs, N factor with 17 miRNAs, LI factor with 2 miRNAs and VI factor with 2 miRNAs. Guo [20] compared miRNA profiles with age, sex, gross pathological types and tumor cells differentiation, revealing that 5 miRNAs were correlated with gross pathological types (fungus and medullar), and 2 miRNAs with tumor cells differentiation (low-, middle- and high-differentiation. But surprisingly, the study found no relationship between miRNA profiles and tobacco and alcohol use, which are 2 independent risk factors of SCC. It was concluded that the specific miRNA profiles have the potential to be promising diagnostic biomarkers for esophageal cancer.

MiRNAs as tumor prognostic biomarkers

The morbidity of esophageal cancer has been steadily increasing due to dietary structure alteration, tobacco and alcohol consumption, inflammation and trauma in the esophagus, as well as genetic factors. Although eradicative surgical resection and chemoradiotherapy have been extensively exploited [111–114], the overall prognosis of patients with esophageal cancer is still poor. However, esophageal cancers diagnosed at the early stage have a good chance of extending a patient’s life. Research on prognostic biomarkers is capable of predicting patient survival and treatment effectiveness, and monitoring tumor recurrence. Many prognostic biomarkers for esophageal cancer have been presented, such as Cyclin D1, Epidermal growth factor receptor, Her-2/Neu, Activated protein C, Transforming growth factor β, Endoglin, Connective tissue growth factor, p53, Bcl-2, NF-kB, Cox-2, E-cadherin, β-catenin, uPA, Matrix metalloproteinase-1,3,7,9, Tissue inhibitor of metalloproteinase, Th1/Th2 balance, C-reactive protein and Parathyroid hormone-related peptide [115]. However, these molecules have intrinsic advantages (eg, lack of specificity and sensitivity), thus the establishment of ideal prognostic biomarkers is able to minimize these disadvantages. The specific miRNA profiles may serve as biomarkers for assessing prognosis of patients. Mathé [107] indicated that low expressed miR375 and miR235 in ADC with BE had a relationship with poor prognosis, concluding that both miRs might be independent prognostic factors for predicting therapy effectiveness in addition to tumor stages and types. It was also demonstrated that highly expressed miR-21, miR-146b, miR-155 and miR-181b in non-cancerous tissues adjacent to cancerous tissues and low expressed miR-223 in cancerous tissues of SCC were associated with poor prognosis; revealing the specific miRNA profiles might be independent prognostic factors of SCC. Ogawa [110] investigated the relationship between miRNA profiles and survival of post-surgery SCC patients, concluding that the patients with highly expressed miR-23a, miR-26a, miR-27b, miR-96, miR-125b and miR-129 showed poor prognosis. In particular, the overexpressed miR-129 might be an independent prognostic factor of post-surgery patients. In the contrary, low expressed miR-103 and miR-107 in SCC showed a strong link to a long survival time. Full advantage has been taken of miRNA profiles of disease prognosis in diversity of tumors, such as non-small-cell lung cancer [116,117], breast cancer [118–121], gastric cancer [102] and hematological tumor [122]. In summary, miRNA profiles may be novel and effective tools for predicting esophageal cancer prognosis in the future.

Circulating miRNAs as diagnostic and prognostic biomarkers of esophageal cancer

Although gastroscopic biopsy and pathological examination are standards for esophageal cancer diagnosis, they have unavoidable disadvantages such as invasiveness, susceptibilitiy to infection, discomfort for patients, easily missed diagnosis and limited physician experience with endoscopy. To some extent these disadvantages, taken together, impede the application of endoscope biopsy in larger-scale populations [123]. The sensitivity and specificity of early tumors diagnosis by endoscope biopsy were low And only 1–2% of early tumors and 15–20% of precancerous lesions in an asymptomatic population aged more than 35 years were diagnosed by endoscope examination [124]. Circulating miRNA may serve as new biomarkers for promotion of sensitivity and specificity of early tumor diagnosis, monitoring tumor progress, evaluating tumor prognosis and detecting tumor recurrence. To date, more than 100 circulating miRNAs have been recognized in healthy individuals. Circulating miRNAs are suitable for biomarkers as evidenced by their being tissue-specific [17,21,125], stable, reproducible and consistent among individuals in the same species [106,126–132]. In addition to the above, high throughput technology, accessible manipulation, low cost and high sensitivity promote the extensive application of circulating miRNAs [133].
Some studies have revealed a relationship between circulating miRNA profiles and varieties of tumors. Ng [134] found that miR-92 was significantly upregulated in plasma of patients with colorectal cancer. The levels of miR-155 of hormone-sensitive and hormone-insensitive breast cancer in serum potentially could predict early breast cancer and evaluate treatment effectiveness and prognosis [135]. Charles [136] also found that circulating miR-21, miR-155 and miR-210 were upregulated in serum of diffuse large B cell lymphoma. It was furthermore found that circulating miR-21 was associated with relapse-free survival. Circulating miRNAs were also specifically and responsively recognized in gastric tumors and pancreatic adenocarcinoma [137,138].

To date, although no articles related to circulating miRNAs in esophageal cancer have been published, the prerequisites of research on circulating miRNAs in esophageal cancer have been satisfied:

- biotechnology promotion, such as MMA [21], bead-based flow-cytometric technology [125], miRNA interfering [139], etc;
- characteristic geographical distribution, sufficient samples and convenient collection;
- establishment of animal models.

Surgical models of gastroesophageal reflux have been established in mice and dogs, subsequently leading to ADC [140–142]. Several carcinogens, such as diethylnitrosamine, were used to induce SCC in mice [143,144]. All in all, circulating miRNAs may predict genesis and turnover of esophageal cancer as accessible and economical biomarkers for diagnosis and prognosis.

**MiRNAs as gene expression regulators in esophageal cancer**

**Effects of miRNAs on biological behaviors of esophageal cancer cells**

The miRNA genes are usually located in or near tumor-related genomic regions. The miRNA profiles of tumor tissues could be regulated by oncogenes, tumor suppressing genes and epigenetics [105,125]. The miRNAs also could affect biological behaviors of tumor cells, including self-renewal, apoptosis, limitless replicative potential,angiogenesis, invasion and metastasis [145]. The miRNAs match 3’ UTR of cancer-related gene transcripts, subsequently silencing cancer-related gene expression.

Annexin A1 (p35), belongs to the calcium- and phospholipid-binding protein family, and associates with arachidonic acid metabolism and epidermal growth factor receptor tyrosine kinase pathway [146]. Annexin A1 was able to prohibit over-proliferation, promote differentiation and apoptosis, regulate cellular migration, membrane trafficking, exocytosis and signal transduction [147,148]. A diversity of tumors was generated partly due to Annexin A1 expression inhibition or loss of ANXA1, such as prostate cancer, breast cancer, esophageal cancer and hematological tumors [149–152]. Luthra [146] revealed that miR-196a matched 3’ UTR of ANXA1 mRNA and disturbed ANXA1 protein biosynthesis, consequently enhancing cancerous proliferation and colony-forming ability and suppressing apoptosis in an esophageal cancer cell line. ANXA1 expression suppression induced by miR-196a also leads to resistance to preoperative chemotherapy in esophageal cancer [153,154]. Maru [155] indicated miR-196a expression could reflect progression of Barrette’s esophagus-dysplasia-adenocarcinoma and malignant transformation of normal esophageal epithelia by associating with 3 target genes – S100A9, SPRR2C and K 5.

Kan [108] reported the expression of miR-106b-25 polycistron, which is an oncogene for tumor transformation, was upregulated in ADC, which might result from elevated copy numbers of MCM7. This highly expressed polycistron was associated with ADC pathogenesis by directly suppressing the CDKN1A expression, which could cause tumor cells ceasing in G1 phase [156], and by negatively regulating pro-apoptotic gene expression, BCL2L11, leading to increased cellular apoptosis [157].

The miR-10b, miR-21 and miR-373 were capable of modulating biological behaviors of squamous carcinoma cells. Lee et al. [158,159] elucidated upregulated miR-373 in SCC was directly associated with tumor suppressing gene LAT32, enhancing tumor cell proliferation, invasion, migration and metastasis. Hiyoshi et al. [160–163] revealed that overexpressed miR-21 interacting with PDCD4, a tumor-suppressing gene transcript, promoted eIF4a transcriptional activity and activator protein-mediated transactivation, as well as inhibited p21 expression, subsequently facilitating malignant transformation of squamous epithelial cells. Tian [164] indicated overexpressed miR-10b in SCC could promote cellular migration and invasion by inhibiting KLF4 expression. KLF4 is a zinc finger protein in the KLF family and plays key roles in regulating cell cycle and cellular differentiation. It is also activated in response to DNA damage, serum starvation and contact inhibition [165].

**Effects of miRNAs on Multi-Drug Resistance (MDR) in esophageal cancer**

Chemotherapy is an important treatment strategy for esophageal cancer. Chemotherapy could down-stage tumors ahead of surgery, inhibit tumor recurrence, and kill metastatic tumor cells [166]. Adjutant and neoadjuvant chemotherapy have been applied clinically; however, a great number of studies have indicated chemotherapy was unable to improve overall survival of patients. Pouliquen [167] revealed no significant difference in overall survival between cisplatin-based chemotherapy and surgery. The incidence of clinically complete response to chemotherapy ranged from 19–58%, whereas that of pathologically complete response was only 2.5–13%, suggesting chemotherapy ineffectiveness [168,169]. It was known that the poor prognosis of esophageal cancer may be partly due to lack of response of tumor cells to chemotherapy. The oncologists observed that SCC was resistant to anti-tumor drugs and developed multiple-drug resistance (MDR), subsequently leading to treatment failure. Wei [120] demonstrated that the EC109/CDDP cell line, which is a MDR SCC cell line, exhibited strong resistance to CDDP, capoblatin, 5-fluorouracil, Taxol, Navelbine, irinotecan and etoposide. This cell line showed alteration in cellular morphology, tumor doubling time and cell cycle distribution compared to EC109. Allen [171,172] proposed a difference of response to neoadjuvant chemotherapy between SCC and ADC (SCC vs. ADC, 61% vs. 20%). Darnton [173] revealed the response to anti-tumor drugs in SCC was
more sensitive than that in ADC (SCC vs. ADC, 74% vs. 30%) by analyzing the different levels of P-glycoprotein (P-GP), which is the product of Multi-Drug Resistance-1 (MDR-1), in biopsy samples from SCC and ADC before and after treatment with Mitomycin, Ifosfamide and cisplatin. There are close links between MDR, P-GP and MDR in esophageal cancer [170–173]. Further studies on molecular mechanisms of MDR are required to discover effective strategies to attenuate or reverse MDR of esophageal cancer. Emerging evidence suggests aberrantly expressed miRNAs might regulate MDR-related genes expression and play key roles in MDR [174,175]. Zhang [176] found that decreased miR-27a expression might enhance the response of anti-tumor drugs to SCC and promote ADR-related tumor cells apoptosis. Bcl-2 and Bax are involved in tumor cells apoptosis and response of tumor cells to chemotherapy [177]. Overexpression of Bcl-2 in a variety of human cancers exhibits poor clinical response to anti-tumor drugs [178]. Decreased miR-27a expression is capable of decreasing Bcl-2 and MDR-1 and increasing Bax, resulting in reversed MDR in esophageal cancer [176]. Hong [179] also discovered that decreased miR-296 expression promotes the response of tumor cells to anti-tumor drugs, probably due to increased apoptotic tumor cells and decreased MDR-1. MDR in esophageal cancer is a complicated process involving a great number of drug resistance-related genes and intracellular signaling pathways. Re-shaping the miRNA profiles is a promising strategy to reverse MDR and enhance the response of esophageal cancer cells to anti-tumor drugs.

**Conclusions**

MicroRNAs are small (~20–24 nucleotides), well conserved, non-coding RNA molecules that either repress translation or promote mRNA degradation based on complementary degree between miRNAs and mRNAs. MiRNA profiles in cancerous tissues or serum have potential to be used as diagnostic and prognostic biomarkers of esophageal cancer. MDR is one of the leading causes for poor prognosis in esophageal cancers. Re-shaping miRNA profiles have potential to reverse MDR and enhance response of esophageal cancer cells to chemotherapy. The relationship between miRNA profiles and MDR of esophageal cancer is particularly complex because miRNAs affect biological behaviors of esophageal cancer cells by targeting hundreds and thousands of tumor-related gene transcripts, while a single tumor-related gene transcript is able to be simultaneously recognized by multiple miRNAs. Progress in understanding the pathogenesis of esophageal cancer appears to be slow, which hinders treatment and prevention. This situation is both a challenge and an opportunity for future research on this disease.

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