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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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Background

Esophageal cancer is the eighth most common cancer and causes the sixth highest cancer-related mortality worldwide [1]. Its morbidity appears to be high in northern China, southeastern Africa and Japan, and is low in western Africa, showing a significant geographic difference [2]. The 5-year survival rate of patients with esophageal cancer, in either the advanced stage or metastasis, is less than 20%. More than 90% of patients could survive past five years if the cancer could be detected and treated early [3,4]. Squamous cell carcinoma (SCC) and adenocarcinoma (ADC) are 2 histological types of esophageal cancer. It is well-known that tobacco and alcohol use are both independent risk factors of SCC, while ADC is susceptible to gastroesophageal reflux and obesity [5,6]. Based on a recent epidemical survey, the morbidity of ADC has increased 6-fold in the past 3 decades [7–9].

MicroRNAs (miRNAs) were initially discovered in *Caenorhabditis elegans* in 1993 [10]. To date, over 9000 miRNAs have been recognized in primates, rodents, birds, fish, worms, flies, plants and viruses [11]. Over 700 human miRNAs have been deposited in the miRBase miRNA registry [12,13]. MiRNA are capable of regulating most of the cell processing procedures (eg, cellular proliferation, differentiation and apoptosis) by inducing mRNA degradation and disturbing protein synthesis [14–16]. Specific miRNAs are capable of activating oncogenes or tumor suppressing genes involved in pathogenesis of tumors [17–21].

The MiRNA profiling platform has been established, providing a strategy to investigate the relationship between miRNA profiles and esophageal cancer diagnosis and prognosis. In this review, we elucidate the biogenesis and function of miRNAs, reveal the relationship between esophageal cancer and specific miRNA profiles, and present specific miRNA profiles as promising diagnostic and prognostic predictors for esophageal cancer.

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 coordinately fine-tune expression of function genes [27–30]. Most mammalian miRNA genes are first transcribed into long primary miRNAs (pre-miRNAs) which are 5' capped and 3' polyadenylated in the nucleus by RNA polymerase II or III [31–33]. The pre-miRNA transcripts comprise 1 or more hairpin structures that are 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 Drosha, which is a member of polymerase RNAase III family in complex with DiGeorge Syndrome Critical Region 8 (DGCR8), which belongs to the double-stranded RNA Binding Domain Protein (dsRBDP) family in the nucleus. Then 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. Drosha engages with DGCR8 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. DGCR8 stretches out 2 dsRNA-binding domains that connect to the junctions between single-stranded and double-stranded regions of the pre-miRNA stem, directing Drosha to crop ~11 bp single strands from pre-miRNA [39,42,43]. Drosha also is composed of 2 RNAase domains that can cleave 5' and 3' 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 Drosha.

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, 35, 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 miRNAs* 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].

Although the canonical pathway is well understood, several pathways associated with miRNA synthesis bypass the canonical pathway, namely the non-canonical pathway. Approximately 40% of miRNA genes reside in introns [32,35]. Mirtrons are defined by the short intronic hairpins

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 a 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 repressing or target mRNAs degradation. Degradation of mRNAs is mediated by mRNAs 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 mRNAs 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 evidences suggested the functional 7-methylguanine cap and polyadenylic tail are prerequisite for miRNA-mediated translation repression [77,78,88]. Kiriakidou [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], Giraldez [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 mRNAs probably were related to increased mRNAs 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, MMA) [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 progestogen 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 sensitivity, highly 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 miR93 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-25 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 clinicopathological 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 deployed [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-κB, 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 those disadvantages. The specific miRNA profiles may serve as biomarkers for assessing prognosis of patients. Mathe [107] indicated that low expressed miR375 and miR233 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-128b 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, susceptibility 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 further 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 mRNAs 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 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 chemoradiotherapy 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 polycystron, which is an oncogene for tumor transformation, was upregulated in ADC, which might result from elevated copy numbers of *MCM7*. This highly expressed polycystron 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 *LATS2*, 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]. Adjuvant 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 etopocide. 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]. Deceased 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 in 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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References:

1. Enzinger PC, Mayer RJ: Esophageal cancer. N Engl J Med, 2003; 349: 2241–52
2. Hollstein MC, Metcalf RA, Welsh JA et al: Frequent mutation of the p53 gene in human esophageal cancer. Proc Natl Acad Sci USA, 1990; 87: 9958–61
3. Headrick JR, Nichols FC III, Miller DL et al: High-grade esophageal dysplasia: long-term survival and quality of life after esophagectomy. Ann Thorac Surg, 2002; 73: 1697–702; discussion 702–3
4. Reed CE: Surgical management of esophageal carcinoma. Oncologist, 1999; 4: 95–105
5. Isono K, Sato H, Nakayama K: Results of a nationwide study on the three-field lymph node dissection of esophageal cancer. Oncology, 1991; 48: 411–20
6. Pisani P, Parkin DM, Bray F et al: Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer, 1999; 83: 18–29
7. Crew KD, Neugut AI: Epidemiology of upper gastrointestinal malignancies. Semin Oncol, 2004; 31: 450–64
8. Brown LM, Devesa SS, Chow WH: Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. J Natl Cancer Inst, 2008; 100: 1184–87
9. Blot WJ, Devesa SS, Kneller RW et al: Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA, 1991; 265: 1287–89
10. Lee RC, Feinbaum RL, Ambros V: The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell, 1993; 75: 843–54
11. Yang Z, Wu J: Small RNAs and development. Med Sci Monit, 2006; 12(7): RA125–29
12. Berezikov E, Guryev V, van de Belt J et al: Phylogenetic shadowing and computational identification of human microRNA genes. Cell, 2005; 120: 21–24
13. Griffiths-Jones S: The microRNA Registry. Nucleic Acids Res, 2004; 32: D109–11
14. Tricoli JV, Jacobson JW: MicroRNA: Potential for Cancer Detection, Diagnosis, and Prognosis. Cancer Res, 2007; 67: 4553–55
15. Miska EA: How microRNAs control cell division, differentiation and death. Curr Opin Genet Dev, 2005; 15: 563–68
16. Zamore PD, Haley B: Ribo-gnome: the big world of small RNAs. Science, 2005; 309: 1519–24
17. Lu J, Getz G, Miska EA et al: MicroRNA expression profiles classify human cancers. Nature, 2005; 435: 834–38
18. Volinia S, Calin GA, Liu CG et al: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA, 2006; 103: 2257–61
19. Feber A, Xi L, Luketich JD et al: MicroRNA expression profiles of esophageal cancer. J Thorac Cardiovasc Surg, 2008; 135: 255–60, discussion 60
20. Guo Y, Chen Z, Zhang L et al: Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. Cancer Res, 2008; 68: 26–33
21. Esquela-Kerscher A, Slack FJ: Oncomirs – microRNAs with a role in cancer. Nat Rev Cancer, 2006; 6: 259–69
22. Lai EC, Tomancak P, Williams RW et al: Computational identification of Drosophila microRNA genes. Genome Biol, 2003; 4: R42
23. Lee Y, Jeon K, Lee JT et al: MicroRNA maturation: stepwise processing and subcellular localization. EMBO J, 2002; 21: 4663–70
24. Luciano DJ, Mirsky H, Vendetti NJ et al: RNA editing of a miRNA precursor. RNA, 2004; 10: 1174–77
25. Lund E, Guttinger S, Calado A et al: Nuclear export of microRNA precursors. Science, 2004; 303: 95–98
26. Lin SL, Miller JD, Ying SY: Intronic microRNA (miRNA). J Biomed Biotechnol, 2006; 2006: 26818
27. Lagos-Quintana M, Rauhut R, Meyer J et al: New microRNAs from mouse and human. RNA, 2003; 9: 175–79
28. Aravin AA, Lagos-Quintana M, Yalcin A et al: The small RNA profile during Drosophila melanogaster development. Dev Cell, 2003; 5: 337–50
29. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004; 116: 281–97
30. Lagos-Quintana M, Rauhut R, Lendeckel W et al: Identification of novel genes coding for small expressed RNAs. Science, 2001; 294: 853–58
31. Borchert GM, Lanier W, Davidson BL: RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol, 2006; 13: 1097–101
32. Cai X, Hagedorn CH, Cullen BR: Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA, 2004; 10: 1957–66
33. Lee Y, Kim M, Han J et al: MicroRNA genes are transcribed by RNA polymerase II. EMBO J, 2004; 23: 4051–60
34. Carthew RW, Sontheimer EJ: Origins and Mechanisms of miRNAs and siRNAs. Cell, 2009; 136: 642–55

35. Kim VN, Han J, Siomi MC: Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol, 2009; 10: 126–39
36. Han J, Lee Y, Yeom KH et al: Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell, 2006; 125: 887–901
37. Zeng Y, Cullen BR: Sequence requirements for micro RNA processing and function in human cells. RNA, 2003; 9: 112–23
38. Zeng Y, Cullen BR: Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences. J Biol Chem, 2005; 280: 27595–603
39. Gregory RI, Yan KP, Amuthan G et al: The Microprocessor complex mediates the genesis of microRNAs. Nature, 2004; 432: 235–40
40. Seitz H, Zamore PD: Rethinking the microprocessor. Cell, 2006; 125: 827–29
41. Denli AM, Tops BB, Plasterk RH et al: Processing of primary microRNAs by the Microprocessor complex. Nature, 2004; 432: 231–35
42. Landthaler M, Yalcin A, Tuschl T: The human DiGeorge syndrome critical region gene 8 and Its D. melanogaster homolog are required for miRNA biogenesis. Curr Biol, 2004; 14: 2162–67
43. Han J, Lee Y, Yeom KH et al: The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev, 2004; 18: 3016–27
44. Kim YK, Kim VN: Processing of intronic microRNAs. EMBO J, 2007; 26: 775–83
45. Morlando M, Ballarino M, Gromak N et al: Primary microRNA transcripts are processed co-transcriptionally. Nat Struct Mol Biol, 2008; 15: 902–9
46. Yi R, Qin Y, Macara IG et al: Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev, 2003; 17: 3011–16
47. Bohnsack MT, Czaplinski K, Gorlich D: Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA, 2004; 10: 185–91
48. Okada C, Yamashita E, Lee SJ et al: A high-resolution structure of the pre-microRNA nuclear export machinery. Science, 2009; 326: 1275–79
49. Lund E, Dahlberg JE: Substrate selectivity of exportin 5 and Dicer in the biogenesis of microRNAs. Cold Spring Harb Symp Quant Biol, 2006; 71: 59–66
50. Bernstein E, Caudy AA, Hammond SM et al: Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature, 2001; 409: 363–66
51. Grishok A, Pasquinelli AE, Conte D et al: Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control C. elegans developmental timing. Cell, 2001; 106: 23–34
52. Hutvagner G, McLachlan J, Pasquinelli AE et al: A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science, 2001; 293: 834–38
53. Ketting RF, Fischer SE, Bernstein E et al: Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev, 2001; 15: 2654–59
54. Knight SW, Bass BL: A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. Science, 2001; 293: 2269–71
55. Chendrimada TP, Gregory RI, Kumaraswamy E et al: TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature, 2005; 436: 740–44
56. Forstemann K, Tomari Y, Du T et al: Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. PLoS Biol, 2005; 3: e236
57. Haase AD, Jaskiewicz L, Zhang H et al: TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. EMBO Rep, 2005; 6: 961–67
58. Jiang F, Ye X, Liu X et al: Dicer-1 and R3D1-L catalyze microRNA maturation in Drosophila. Genes Dev, 2005; 19: 1674–79
59. Saito K, Ishizuka A, Siomi H et al: Processing of pre-microRNAs by the Dicer-1-Loquacious complex in Drosophila cells. PLoS Biol, 2005; 3: e235
60. Lau NC, Lim LP, Weinstein EG et al: An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science, 2001; 294: 858–62
61. Cullen BR: Transcription and processing of human microRNA precursors. Mol Cell, 2004; 16: 861–65
62. Siomi H, Siomi MC: On the road to reading the RNA-interference code. Nature, 2009; 457: 396–404
63. Khvorova A, Reynolds A, Jayasena SD: Functional siRNAs and miRNAs exhibit strand bias. Cell, 2003; 115: 209–16
64. Schwarz DS, Hutvagner G, Du T et al: Asymmetry in the assembly of the RNAi enzyme complex. Cell, 2003; 115: 199–208
65. Ghildiyal M, Xu J, Seitz H et al: Sorting of Drosophila small silencing RNAs partitions microRNA* strands into the RNA interference pathway. RNA, 2010; 16: 43–56
66. Okamura K, Phillips MD, Tyler DM et al: The regulatory activity of microRNA* species has substantial influence on microRNA and 3' UTR evolution. Nat Struct Mol Biol, 2008; 15: 354–63
67. Okamura K, Hagen JW, Duan H et al: The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell, 2007; 130: 89–100
68. Ruby JG, Jan CH, Bartel DP: Intronic microRNA precursors that bypass Drosha processing. Nature, 2007; 448: 83–86
69. Ender C, Krek A, Friedlander MR et al: A human snoRNA with microRNA-like functions. Mol Cell, 2008; 32: 519–28
70. Matera AG, Terns RM, Terns MP: Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. Nat Rev Mol Cell Biol, 2007; 8: 209–20
71. Babiarz JE, Ruby JG, Wang Y et al: Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. Genes Dev, 2008; 22: 2773–85
72. Pillai RS, Bhattacharyya SN, Filipowicz W: Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol, 2007; 17: 118–26
73. Peters L, Meister G: Argonaute proteins: mediators of RNA silencing. Mol Cell, 2007; 26: 611–23
74. Bartel DP: MicroRNAs: target recognition and regulatory functions. Cell, 2009; 136: 215–33
75. Orom UA, Nielsen FC, Lund AH: MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell, 2008; 30: 460–71
76. Krol J, Loedige I, Filipowicz W: The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet, 2010; 11: 597–610
77. Humphreys DT, Westman BJ, Martin DI et al: MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. Proc Natl Acad Sci USA, 2005; 102: 16961–66
78. Pillai RS, Bhattacharyya SN, Artus CG et al: Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science, 2005; 309: 1573–76
79. Kiriakidou M, Tan GS, Lamprinaki S et al: An mRNA m7G cap binding-like motif within human Ago2 represses translation. Cell, 2007; 129: 1141–51
80. Mathonnet G, Fabian MR, Svitkin YV et al: MicroRNA inhibition of translation initiation *in vitro* by targeting the cap-binding complex eIF4F. Science, 2007; 317: 1764–67
81. Wakiyama M, Takimoto K, Ohara O et al: Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. Genes Dev, 2007; 21: 1857–62
82. Ding XC, Grosshans H: Repression of C. elegans microRNA targets at the initiation level of translation requires GW182 proteins. EMBO J, 2009; 28: 213–22
83. Seggerson K, Tang L, Moss EG: Two genetic circuits repress the Caenorhabditis elegans heterochronic gene lin-28 after translation initiation. Dev Biol, 2002; 243: 215–25
84. Maroney PA, Yu Y, Fisher J et al: Evidence that microRNAs are associated with translating messenger RNAs in human cells. Nat Struct Mol Biol, 2006; 13: 1102–7
85. Nottrott S, Simard MJ, Richter JD: Human let-7a miRNA blocks protein production on actively translating polyribosomes. Nat Struct Mol Biol, 2006; 13: 1108–14
86. Petersen CP, Bordeleau ME, Pelletier J et al: Short RNAs repress translation after initiation in mammalian cells. Mol Cell, 2006; 21: 533–42
87. Eulalio A, Huntzinger E, Izaurralde E: Getting to the root of miRNA-mediated gene silencing. Cell, 2008; 132: 9–14
88. Wang B, Love TM, Call ME et al: Recapitulation of short RNA-directed translational gene silencing *in vitro*. Mol Cell, 2006; 22: 553–60
89. Thermann R, Hentze MW: Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. Nature, 2007; 447: 875–78
90. Behm-Ansmant I, Rehwinkel J, Doerks T et al: mRNA degradation by miRNAs and GW182 requires both CCR4: NOT deadenylase and DCP1: DCP2 decapping complexes. Genes Dev, 2006; 20: 1885–98
91. Giraldez AJ, Mishima Y, Rihel J et al: Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. Science, 2006; 312: 75–79

92. Wu L, Fan J, Belasco JG: MicroRNAs direct rapid deadenylation of mRNA. Proc Natl Acad Sci USA, 2006; 103: 4034–39
93. Fabian MR, Sonenberg N, Filipowicz W: Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem, 2010; 79: 351–79
94. Eulalio A, Tritschler F, Izaurralde E: The GW182 protein family in animal cells: new insights into domains required for miRNA-mediated gene silencing. RNA, 2009; 15: 1433–42
95. Bagga S, Bracht J, Hunter S et al: Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell, 2005; 122: 553–63
96. Lim LP, Lau NC, Garrett-Engele P et al: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature, 2005; 433: 769–73
97. Aleman LM, Doench J, Sharp PA: Comparison of siRNA-induced off-target RNA and protein effects. RNA, 2007; 13: 385–95
98. Liu CG, Calin GA, Meloon B et al: An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. Proc Natl Acad Sci USA, 2004; 101: 9740–44
99. Fabbri M: miRNAs as molecular biomarkers of cancer. Expert Rev Mol Diagn, 2010; 10: 435–44
100. Yanaihara N, Caplen N, Bowman E et al: Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell, 2006; 9: 189–98
101. Iorio MV, Ferracin M, Liu CG et al: MicroRNA gene expression deregulation in human breast cancer. Cancer Res, 2005; 65: 7065–70
102. Ueda T, Volinia S, Okumura H et al: Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. Lancet Oncol, 2010; 11: 136–46
103. Porkka KP, Pfeiffer MJ, Waltering KK et al: MicroRNA expression profiling in prostate cancer. Cancer Res, 2007; 67: 6130–35
104. Cummins JM, He Y, Leary RJ et al: The colorectal microRNAome. Proc Natl Acad Sci USA, 2006; 103: 3687–92
105. Calin GA, Liu CG, Sevignani C et al: MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA, 2004; 101: 11755–60
106. Mitchell PS, Parkin RK, Kroh EM et al: Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA, 2008; 105: 10513–18
107. Mathe EA, Nguyen GH, Bowman ED et al: MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. Clin Cancer Res, 2009; 15: 6192–200
108. Kan T, Sato F, Ito T et al: The miR-106b-25 polycistron, activated by genomic amplification, functions as an oncogene by suppressing p21 and Bim. Gastroenterology, 2009; 136: 1689–700
109. Yang H, Gu J, Wang KK et al: MicroRNA expression signatures in Barrett's esophagus and esophageal adenocarcinoma. Clin Cancer Res, 2009; 15: 5744–52
110. Ogawa R, Ishiguro H, Kuwabara Y et al: Expression profiling of microRNAs in human esophageal squamous cell carcinoma using RT-PCR. Med Mol Morphol, 2009; 42: 102–9
111. Rouvelas I, Zeng W, Lindblad M et al: Survival after surgery for oesophageal cancer: a population-based study. Lancet Oncol, 2005; 6: 864–70
112. Prasad GA, Wu TT, Wigle DA et al: Endoscopic and surgical treatment of mucosal (T1a) esophageal adenocarcinoma in Barrett's esophagus. Gastroenterology, 2009; 137: 815–23
113. Shaheen NJ, Sharma P, Overholt BF et al: Radiofrequency ablation in Barrett's esophagus with dysplasia. N Engl J Med, 2009; 360: 2277–88
114. Lagergren J, Lagergren P: Oesophageal cancer. BMJ, 2010; 341: c6280
115. Lagarde SM, ten Kate FJ, Richel DJ et al: Molecular prognostic factors in adenocarcinoma of the esophagus and gastroesophageal junction. Ann Surg Oncol, 2007; 14: 977–91
116. Yu SL, Chen HY, Chang GC et al: MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell, 2008; 13: 48–57
117. Garofalo M, Di Leva G, Romano G et al: miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell, 2009; 16: 498–509
118. Zhu S, Wu H, Wu F et al: MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res, 2008; 18: 350–59
119. Yan LX, Huang XF, Shao Q et al: MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA, 2008; 14: 2348–60
120. Huang Q, Gumireddy K, Schrier M et al: The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol, 2008; 10: 202–10
121. Diaz LK, Zhou X, Wright ET et al: CD44 expression is associated with increased survival in node-negative invasive breast carcinoma. Clin Cancer Res, 2005; 11: 3309–14
122. Calin GA, Ferracin M, Cimmino A et al: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med, 2005; 353: 1793–801
123. Zhou SL, Wang LD: Circulating microRNAs: novel biomarkers for esophageal cancer. World J Gastroenterol, 2010; 16: 2348–54
124. Wang LD, Zhou Q, Feng CW et al: Intervention and follow-up on human esophageal precancerous lesions in Henan, northern China, a high-incidence area for esophageal cancer. Gan To Kagaku Ryoho, 2002; 29(Suppl.1): 159–72
125. Calin GA, Croce CM: MicroRNA signatures in human cancers. Nat Rev Cancer, 2006; 6: 857–66
126. Lo KW, Lo YM, Leung SF et al: Analysis of cell-free Epstein-Barr virus associated RNA in the plasma of patients with nasopharyngeal carcinoma. Clin Chem, 1999; 45: 1292–94
127. Kopreski MS, Benko FA, Kwak LW et al: Detection of tumor messenger RNA in the serum of patients with malignant melanoma. Clin Cancer Res, 1999; 5: 1961–65
128. Chen XQ, Bonnefoi H, Pelte MF et al: Telomerase RNA as a detection marker in the serum of breast cancer patients. Clin Cancer Res, 2000; 6: 3823–26
129. Fabian TJ, Dew MA, Pollock BG et al: Endogenous concentrations of DHEA and DHEA-S decrease with remission of depression in older adults. Biol Psychiatry, 2001; 50: 767–74
130. Lo YM, Tsui NB, Chiu RW et al: Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med, 2007; 13: 218–23
131. Swarup V, Rajeswari MR: Circulating (cell-free) nucleic acids – a promising, non-invasive tool for early detection of several human diseases. FEBS Lett, 2007; 581: 795–99
132. Tsui NB, Ng EK, Lo YM: Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem, 2002; 48: 1647–53
133. Chen C, Ridzon DA, Broomer AJ et al: Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res, 2005; 33: e179
134. Ng EK, Chong WW, Jin H et al: Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut, 2009; 58: 1375–81
135. Zhu W, Qin W, Atasoy U et al: Circulating microRNAs in breast cancer and healthy subjects. BMC Res Notes, 2009; 2: 89
136. Lawrie CH, Gal S, Dunlop HM et al: Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol, 2008; 141: 672–75
137. Wang J, Chen J, Chang P et al: MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prev Res (Phila), 2009; 2: 807–13
138. Tsujiura M, Ichikawa D, Komatsu S et al: Circulating microRNAs in plasma of patients with gastric cancers. Br J Cancer, 2010; 102: 1174–79
139. Koga K, Matsumoto K, Akiyoshi T et al: Purification, characterization and biological significance of tumor-derived exosomes. Anticancer Res, 2005; 25: 3703–7
140. Hao J, Liu B, Yang CS et al: Gastroesophageal reflux leads to esophageal cancer in a surgical model with mice. BMC Gastroenterol, 2009; 9: 59
141. Babu A, Meng X, Banerjee AM et al: Secretory phospholipase A2 is required to produce histologic changes associated with gastroduodenal reflux in a murine model. J Thorac Cardiovasc Surg, 2008; 135: 1220–27
142. McMahon RL, Ali A, Chekan EG et al: A canine model of gastroesophageal reflux disease (GERD). Surg Endosc, 2002; 16: 67–74
143. Zgodzinski W, Zinkiewicz K, Juskiewicz W et al: Diethylnitrosamine may induce esophageal dysplasia after local intramural administration. Rocz Akad Med Bialymst, 2003; 48: 48–51
144. Andrejevic S, Savary JF, Fontolliet C et al: 7,12-dimethylbenz[a]anthracene-induced 'early' squamous cell carcinoma in the Golden Syrian hamster: evaluation of an animal model and comparison with 'early' forms of human squamous cell carcinoma in the upper aero-digestive tract. Int J Exp Pathol, 1996; 77: 7–14
145. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell, 2000; 100: 57–70
146. Luthra R, Singh RR, Luthra MG et al: MicroRNA-196a targets annexin A1: a microRNA-mediated mechanism of annexin A1 downregulation in cancers. Oncogene, 2008; 27: 6667–78

147. Parente L, Solito E: Annexin 1: more than an anti-phospholipase protein. Inflamm Res, 2004; 53: 125–32

148. Gerke V, Moss SE: Annexins: from structure to function. Physiol Rev, 2002; 82: 331–71

149. Kang JS, Calvo BF, Maygarden SJ et al: Dysregulation of annexin I protein expression in high-grade prostatic intraepithelial neoplasia and prostate cancer. Clin Cancer Res, 2002; 8: 117–23

150. Shen D, Nooraie F, Elshimali Y et al: Decreased expression of annexin A1 is correlated with breast cancer development and progression as determined by a tissue microarray analysis. Hum Pathol, 2006; 37: 1583–91

151. Paweletz CP, Ornstein DK, Roth MJ et al: Loss of annexin 1 correlates with early onset of tumorigenesis in esophageal and prostate carcinoma. Cancer Res, 2000; 60: 6293–97

152. Vishwanatha JK, Salazar E, Gopalakrishnan VK: Absence of annexin I expression in B-cell non-Hodgkin's lymphomas and cell lines. BMC Cancer, 2004; 4: 8

153. Xia SH, Hu LP, Hu H et al: Three isoforms of annexin I are preferentially expressed in normal esophageal epithelia but down-regulated in esophageal squamous cell carcinomas. Oncogene, 2002; 21: 6641–48

154. Luthra R, Wu TT, Luthra MG et al: Gene expression profiling of localized esophageal carcinomas: association with pathologic response to preoperative chemoradiation. J Clin Oncol, 2006; 24: 259–67

155. Maru DM, Singh RR, Hannah C et al: MicroRNA-196a is a potential marker of progression during Barrett's metaplasia-dysplasia-invasive adenocarcinoma sequence in esophagus. Am J Pathol, 2009; 174: 1940–48

156. Gartel AL, Radhakrishnan SK: Lost in transcription: p21 repression, mechanisms, and consequences. Cancer Res, 2005; 65: 3980–85

157. O'Connor L, Strasser A, O'Reilly LA et al: Bim: a novel member of the Bcl-2 family that promotes apoptosis. EMBO J, 1998; 17: 384–95

158. Lee KH, Goan YG, Hsiao M et al: MicroRNA-373 (miR-373) post-transcriptionally regulates large tumor suppressor, homolog 2 (LATS2) and stimulates proliferation in human esophageal cancer. Exp Cell Res, 2009; 315: 2529–38

159. Li Y, Pei J, Xia H et al: Lats2, a putative tumor suppressor, inhibits G1/S transition. Oncogene, 2003; 22: 4398–405

160. Hiyoshi Y, Kamohara H, Karashima R et al: MicroRNA-21 regulates the proliferation and invasion in esophageal squamous cell carcinoma. Clin Cancer Res, 2009; 15: 1915–22

161. Yang HS, Jansen AP, Nair R et al: A novel transformation suppressor, Pdcd4, inhibits AP-1 transactivation but not NF-kappaB or ODC transactivation. Oncogene, 2001; 20: 669–76

162. Yang HS, Jansen AP, Komar AA et al: The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. Mol Cell Biol, 2003; 23: 26–37

163. Goke R, Barth P, Schmidt A et al: Programmed cell death protein 4 suppresses CDK1/cdc2 via induction of p21(Waf1/Cip1). Am J Physiol Cell Physiol, 2004; 287: C1541–46

164. Tian Y, Luo A, Cai Y et al: MicroRNA-10b promotes migration and invasion through KLF4 in human esophageal cancer cell lines. J Biol Chem, 2010; 285: 7986–94

165. Shields JM, Christy RJ, Yang VW: Identification and characterization of a gene encoding a gut-enriched Kruppel-like factor expressed during growth arrest. J Biol Chem, 1996; 271: 20009–17

166. Campbell NP, Villaflor VM: Neoadjuvant treatment of esophageal cancer. World J Gastroenterol, 2010; 16: 3793–803

167. Pouliquen X, Levard H, Hay JM et al: 5-Fluorouracil and cisplatin therapy after palliative surgical resection of squamous cell carcinoma of the esophagus. A multicenter randomized trial. French Associations for Surgical Research. Ann Surg, 1996; 223: 127–33

168. Schlag PM: Randomized trial of preoperative chemotherapy for squamous cell cancer of the esophagus. The Chirurgische Arbeitsgemeinschaft Fuer Onkologie der Deutschen Gesellschaft Fuer Chirurgie Study Group. Arch Surg, 1992; 127: 1446–50

169. Maipang T, Vasinanukorn P, Petpichetchian C et al: Induction chemotherapy in the treatment of patients with carcinoma of the esophagus. J Surg Oncol, 1994; 56: 191–97

170. Wen J, Zheng B, Hu Y et al: Establishment and biological analysis of the EC109/CDDP multidrug-resistant esophageal squamous cell carcinoma cell line. Oncol Rep, 2009; 22: 65–71

171. Allen SM, Duffy JP, Walker SJ et al: A phase II study of mitomycin, ifosfamide and cisplatin in operable and inoperable squamous cell carcinoma of the oesophagus. Clin Oncol (R Coll Radiol), 1994; 6: 91–95

172. Allen SM, Duffy JP, Darnton SJ et al: Phase II study of mitomycin, ifosfamide and cisplatin in adenocarcinoma of the oesophagus. Cancer Chemother Pharmacol, 1996; 37: 496–98

173. Darnton SJ, Jenner K, Steyn RS et al: Lack of correlation of P-glycoprotein expression with response to MIC chemotherapy in oesophageal cancer. J Clin Pathol, 1995; 48: 1064–66

174. Tsai WC, Hsu PW, Lai TC et al: MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. Hepatology, 2009; 49: 1571–82

175. Cordes KR, Srivastava D: MicroRNA regulation of cardiovascular development. Circ Res, 2009; 104: 724–32

176. Zhang H, Li M, Han Y et al: Down-regulation of miR-27a might reverse multidrug resistance of esophageal squamous cell carcinoma. Dig Dis Sci, 2010; 55: 2545–51

177. Kang MH, Reynolds CP: Bcl-2 inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy. Clin Cancer Res, 2009; 15: 1126–32

178. Asakura T, Ohkawa K: Chemotherapeutic agents that induce mitochondrial apoptosis. Curr Cancer Drug Targets, 2004; 4: 577–90

179. Hong L, Han Y, Zhang H et al: The prognostic and chemotherapeutic value of miR-296 in esophageal squamous cell carcinoma. Ann Surg, 2010; 251: 1056–63