MicroRNAs as oncogenes or tumour suppressors in oesophageal cancer: potential biomarkers and therapeutic targets

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Abstract

MicroRNAs are a class of small, non-coding RNAs that can negatively regulate protein-coding genes, and are associated with almost all known physiological and pathological processes, especially cancer. The number of studies documenting miRNA expression patterns in malignancy continues to expand rapidly, with continuously gained critical information regarding how aberrantly expressed miRNAs may contribute to carcinogenesis. miRNAs can influence cancer pathogenesis, playing a potential role as either oncogenes or tumour suppressors. Recently, several miRNAs have been reported to exert different regulatory functions in oesophageal cancer – the carcinoma typically arising from the epithelial lining of the oesophagus. These miRNAs also have potential clinical applications towards developing biomarkers or targets for possible use in diagnosis or therapy in oesophageal cancer. In this review, we have summarized the two (oncogenic or tumour suppressive) roles of miRNAs here, and their applications as potential biomarkers or therapeutic targets, which may illuminate future treatment for oesophageal cancer.

Introduction

MicroRNAs (miRNAs), are an endogenous class of 17–25 nucleotide-long non-coding RNAs, that can play critical roles in regulation of gene expression (1). The first identification of an miRNA documented involvement of lin4 in cell differentiation of *Caenorhabditis elegans* (2). Since then, more studies have rapidly been expanding the number of identified miRNAs, thus providing better understanding of their roles in human disease, specially cancer.

The majority of miRNAs are initially transcribed by RNA polymerase II as long primary transcripts characterized by hairpin structure, pri-miRNA, which contains stem-loop structures, the 5'-end cap and the 3'-poly (A) tail (3).

The first step in miRNA processing occurs in the nucleus, facilitated by endonuclease RNA polymerase III (Drosha) and double-stranded RNA-binding domain protein DGCR8. Drosha cleaves strands of the pri-miRNA transcript in a staggered manner to produce a stem-loop precursor molecule (pre-miRNA) approximately 70 nucleotides in length (4). These pre-miRNA molecules are then transported to the cytoplasm, this being mediated by nuclear transport receptor Exportin-5 and nuclear protein Ran-GTP. Subsequently, pre-miRNA is loaded to a protein complex containing cytoplasmic RNase III, endonuclease Dicer and trans-activating response RNA-binding protein, to produce the single-stranded mature miRNA, which can incorporate into an RNA-induced silencing complex (RISC). Dicer complex facilitates following processing (5). Subsequently, it interacts with trans-activating response RNA-binding protein thus generating an miRNA/miRNA* duplex. Processing appears to be coupled to assembly of the mature single strand miRNA into RISC, which contains some key proteins, such as Argonaut 2 (AGO2) (6). In

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Figure 1. Biogenesis of microRNAs. MiRNAs are transcribed by RNA polymerase II into long pri-miRNA, which can be recognized and cleaved in the nucleus by Drosha, resulting in a hairpin precursor form called pre-miRNA. Then, the pre-miRNA is exported from the nucleus to the cytoplasm by Exportin 5 and is further processed by Dicer, to produce a duplex molecule containing single-stranded mature miRNA and a miRNA* fragment. The miRNA:miRNA* duplex is unwound by helicase and the miRNA* fragment is degraded, whereas the mature miRNA molecule binds to an Ago protein and incorporates into the RNA-induced silencing complex. Lastly, mature miRNA causes RNA-induced silencing complex to cleave the mRNA or induce translational repression, depending on the degree of complementarity between this miRNA and its target.

mammals, AGO2 protein complex cooperates in Dicer-mediated processing of some pre-miRNAs, yielding a further intermediate processing product, AGO2-cleaved precursor miRNA (7). At the post-transcriptional level, mature miRNA regulates gene expression, thereby tethering RISC by binding, generally through partial complementarity, to target mRNAs and primarily leading to mRNA degradation or translation inhibition (7). Additionally, miRNAs can interact with 3'-untranslated regions (3'-UTRs) of target mRNA through imperfect base pairing (Fig. 1).

As far as we know, miRNA expression can be deregulated in cancer by a variety of mechanisms including amplification, deletion, and mutation as well as by epigenetic silencing, and a number of studies has shown that miRNAs are involved in initiation and progression of cancer. The link between miRNAs and cancer was first highlighted, in chronic lymphocytic leukaemia, by two tumour suppressive miRNAs, miR-15a and miR-16-1, located on chromosome 13, with usual down-regulation (8). Then, the first oncogenic miR-155 was found to be frequently overexpressed in haematopoietic malignancies (9). After identification of miR-155, aberrant expression patterns of miRNAs have been found to be associated with tumorigenesis, in which miRNAs may act as either oncogenes or tumour suppressors. On the one hand, miRNAs amplified or overexpressed in cancer, can be regarded in the same way as oncogenes. Although the list of miRNAs that function similarly to oncogenes is short, evidence of

their role in cancer is very strong. On the other hand, as with protein-coding genes, miRNAs can act as tumour suppressors when their loss of function can initiate or contribute to malignant transformation of normal cells. In 2008, the first report concerning miRNA expression profiles in oesophageal cancer was published. Since then, more studies have focused on the relationship between oncogenic/tumour suppressive miRNAs and oesophageal cancer.

Oesophageal cancer, one of the eight most common cancers worldwide and of the six most common causes of cancer death, can mainly be divided into two types, adenocarcinoma and squamous cell carcinoma (10). Oesophageal adenocarcinoma (EAC) usually develops in the lower portion of the oesophagus and arises after changes associated with Barrett's oesophagus (BE), while oesophageal squamous cell carcinoma (ESCC) originates in squamous epithelium of the oesophagus and is related to tobacco consumption (11,12). EAC is the most rapidly increasing cancer in western countries, with a dramatic rise in incidence observed over the last 20 years (13). BE, the precursor condition for EAC, proceeds from dysplastic stages through low-grade dysplasia (LGD) to high-grade dysplasia (HGD) on to adenocarcinoma (14). In contrast, ESCC is dominant in developing countries, particularly in East Asia where tobacco use is still widespread. Incidence levels for this subtype are relatively stable from a global perspective, but rates in Western countries have fallen (15). Prognoses for affected patients, often diagnosed at later stages

of ESCC, are unsatisfactory despite development of therapeutic options such as surgery, chemotherapy and radiotherapy (16). Although incidence of oesophageal carcinoma (EC) has been reduced in recent decades, 5-year survival and mortality have not changed significantly; thus the disease poses challenges in early diagnosis, staging and prognosis, as well as for selection and delivery of optimal therapy modality. There is clearly need for better understanding of molecular characteristics of the disease, for identification of new molecular markers. Hitherto, an avalanche of research has established that miRNAs can provide a new tool for early clinical diagnosis, prognostic judgment and gene therapy for EC (17). Follow-up studies on miRNAs may also lead to improved survival rates and to reduce mortality levels of EC patients (18).

In this review, we summarize both oncogenic and tumour suppressive miRNAs in oesophageal cancer, with their potentials in prognosis, diagnosis and therapeutics, which may shed new light on future development of its treatment.

Oncogenic microRNAs in oesophageal cancer

Oncogenic miR-21 can be over-expressed in ESCC and is correlated with development of cancer; its down-regulation can inhibit cell proliferation and induce apoptosis, with identified direct targets such as FASL, TIMP3 and RECK (19). The novel arene Ru (II) drug, Rawq01, may abrogate effects of miR-21 on the PTEN/AKT signalling pathway, as Rawq01 may upregulate PTEN expression through miR-21 inhibition, then suppress the PI3K-AKT pathway (20). Also, over-expression of miR-31 and miR-21 may be accompanied by down-regulation of their respective tumour suppressor targets PPP2R2A and PDCD4 in ESCC. miR-31 and miR-21 levels are directly associated with the appearance of ESCC (21). Up-regulation of miR-92a significantly connects with status of lymph node metastasis (LNM) and TNM stage in ESCC cells, but only modulates migration and invasion, not apoptosis not cell proliferation. Furthermore, miR-92a may directly target the 3'-UTR of tumour metastasis suppressor CDH1 to repress its expression. Restoration of miR-92a-resistant CDH1 expression in cells can recover pro-metastatic activity of miR-92a (22). A putative binding site for miR-196a exists in the 3'-UTR of cancer promoter RAP1A, and genetic variant, rs6573 A→C, is also present in this binding region. SNP rs6573 A→C change can interfere with interaction of miR-196a binding to 3'-UTR of RAP1A, leading to higher constitutive expression of RAP1A in the majority of ESCC cells (23). miR-200c influences sensitivity to chemotherapy and radiotherapy

in ESCC, and patients with high expression of serum miR-200c may suffer higher risk of death than those with its low expression; thereby serum level of miR-200c can be useful for predicting responses to chemotherapy and prognosis of patients with oesophageal cancer (24). Increased levels of miR-221/222 have also been observed in EAC. Exposure of oesophageal cells to bile acids may activate farnesoid X receptor and upregulate levels of miR-221/222, by reducing levels of p27^{Kip1} and promoting degradation of CDX2 (25). Moreover, miR-223 can directly target PARP1 during the BE-dysplasia-EAC sequence, and the epithelial compartment can exclusively express miR-223 (26).

A member of the LATS family, LATS2 is implicated in regulation of the cell cycle and apoptosis. It has been confirmed that miR-373 can inhibit protein expression of LATS2, thus leading to activation of ESCC cells (27). Further investigation has shown that expression levels of miR-577 and tumour-associated antigen testis specific 10 (TSGA10) are negatively correlated with each other in ESCC cells; thereby miR-577 can regulate TSGA10 and in turn affect cell proliferation. miR-577/TSGA10 axis activation is accompanied by inactivation of the p53 pathway, which is obligatory for progression of ESCCs with miR-577/TSGA10 axis activation (28) (Table 1).

Tumour suppressive microRNAs in oesophageal cancer

The role of let-7 is important in regulating high mobility group A2 (HMGA2) protein expression to inhibit cell proliferation in ESCC. Also here, HMGA2 is negatively regulated by let-7 at the post-transcriptional level (29). Furthermore, expression of let-7 in oesophageal cancer can be used to predict response to cisplatin-based chemotherapy, as transfection of let-7c can directly repress the cisplatin-activated interleukin (IL)-6/STAT3 pro-survival pathway then increase level of apoptosis after exposure to cisplatin (30). A novel mechanism of miR-34a regulation on ESCC is that NF-κB can elevate miR-34a expression levels through directly binding to its promoter, and wild-type p53 is responsible for NF-κB-mediated miR-34a transcriptional activity. This might reveal new perspectives for roles of miR-34a and NF-κB in oesophageal cancer progression (31). Expression level of miR-98 and miR-214 is inversely correlated with enhancer of zeste homolog 2 (EZH2) protein expression and clinical features such as pathological grade, tumour stage and LMN, suggesting that increased expression of miR-98 and miR-214 might prevent metastasis of human ESCC by reducing accumulation of EZH2 protein (32). Overexpression of miR-99a and

Table 1. MicroRNAs function as either oncogenes or tumour suppressors in oesophageal cancer

MicroRNA	Oncogene (OG)/tumour suppressor (TS)	Target	Function	Reference
let-7a	TS	HMGA2	Inhibition of cell proliferation and induction of apoptosis Repression of cisplatin-activated interleukin (IL)-6/STAT3 pro-survival pathway	29,30
miR-21	OG	FASL, TIMP3, RECK, PTEN	Inhibition of cell growth and cell invasion, as well as induction of cells to apoptosis. Prevention of the PTEN/AKT signalling pathway	19,20
miR-31	OG	PPP2R2A, PDCD4	Downregulation of tumour suppressor targets PPP2R2A and PDCD4	21
miR-34a	TS	NF-κB	The elevation of miR-34a expression levels by NF-κB	31
miR-92a	OG	CDH1	Modulation of cell migration and invasion, but not apoptosis and proliferation	22
miR-98/miR-214	TS	EZH2	Promotion of ESCC metastasis <i>via</i> inducing accumulation of EZH2 protein	32
miR-99a/miR-100	TS	mTOR	Inhibition of cell proliferation by inducing apoptosis	33
miR-129-2	TS	SOX4	Suppression of cell migration and proliferation	34
miR-133a	TS	CD47	Inhibition of tumorigenesis and growth	35,36
miR-138	TS	FSCN1, MMP14	Decrease of both mRNA and protein levels of FSCN1 and MMP14	37
miR-143	TS	FLOT1, FLOT2, Caveolin-1	Reduction of the aggressive phenotype of ESCC cells	38
miR-150	TS	ERK-5 ZEB1	Induction of growth suppression and apoptosis Inhibition of tumorigenicity and tumour growth	39
miR-196a	OG	RAP1A	Induction of the higher constitutive expression of RAP1A	23
miR-200b	TS	Kindlin-2	Regulation of tumour invasiveness and actin cytoskeleton Decreases of cell protrusion and focal adhesion formation Reduction of cell spreading and invasiveness/migration	40
miR-203	TS	Bim-1 LIM LASP1	Control over cell proliferation and self-renewals Association with LASKP1 to influence the development of ESCC cells	41,42
miR-221/ miR-222	OG	p27 ^{Kip1} CDX2	Reduction of p27 ^{Kip1} level and degradation of CDX2	25
miR-223	OG	PARP1	Induction of migratory and invasive potential	26
miR-373	OG	LAST2	Inhibition of LAST protein expression	27
miR-375	TS	LDHB AEG-1/MTDH	Regulation on LDHB and AEG-1/MTDH	43
miR-429	TS	Bcl-2 SP1	Inhibition of invasion and promotion of apoptosis	44
miR-518b	TS	Rap1b	Suppression of cell proliferation by inducing apoptosis and repressing the invasion	45
miR-577	OG	TSGA10	Inactivation of the p53 pathway or the Rb pathway or both	28
miR-655	TS	PTTG1	Inhibition of ESCC cell invasiveness	46

miR-100 *via* transient transfection of corresponding precursor molecules can inhibit cell proliferation by inducing apoptosis in ESCC cells. It can also suppress expression of mTOR by directly targeting 3'-UTR of mTOR in a post-transcriptional manner. These results may indicate that miR-99a and miR-100 have negative influence on cell proliferation by suppressing mTOR in (33). Expression of miR-129-2 is down-regulated when SOX4 expression is elevated. Restoration of miR-129-2 by transfection with miRNA expression plasmid leads to reduction in SOX4 expression, accompanied by reduced migration and proliferation of oesophageal cancer cells (34). CD47, which can inhibit phagocytosis, is directly

regulated by miR-133a, whose over-expression is correlated with poor prognosis; alternatively, it can significantly inhibit carcinogenesis (35). As invadopodia-related proteins, both actin-binding protein Fascin homolog 1 (FSCN1) and matrix metalloproteinase 14 (MMP14) are reduced by miR-133a for both mRNA and protein levels. Also, knockdown of FSCN1 or MMP14 and transfection of an miR-133a mimic may inhibit ESCC cell proliferation or invasion (36). Furthermore, miR-138 is down-regulated and is inversely correlated with tumour progression. It has been demonstrated that down-regulation of miR-138 enhances, whereas up-regulation of miR-138 reduces, the aggressive phenotype

of ESCC cells. Silencing miR-138 can promote K63-linked polyubiquitination of NF- κ B signalling, and mediate TRAF2 as well as RIP1. Down-regulation of miR-138 also induces lipid raft formation *via* up-regulating multiple components of lipid rafts, such as FLOT1, FLOT2 and caveolin-1 (37). Enforced expression of tumour suppressor miR-143 can induce suppression of population growth and apoptosis of ESCC cells, while rescue of miR-143 significantly suppresses their migration and invasion capabilities. Moreover, functions of miR-143 are mediated by inhibition of extracellular signal-regulated kinase-5 activity (38). Low expression of miR-150 in ESCC may contribute to their malignant potential, such as tumour depth, LNM, lymphatic invasion, venous invasion, clinical staging, and poor prognosis, among which *ZEB1*, an epithelial mesenchymal transition inducer, has been regarded as a target gene. Also, miR-150 can induce MET-like changes in TE-8 cells through E-cadherin expression, epithelial morphology, and suppression of migration ability, whence it can significantly inhibit tumour growth (39). In ESCC, miR-200b targets Kindlin-2, which is implicated in regulation of tumour invasiveness and status of the actin cytoskeleton. Enforced expression of miR-200b mimics may result in reduction of Kindlin-2, whereas transfection of miR-200b inhibitors induces Kindlin-2 expression. Furthermore, transfection of miR-200b mimic or knockdown of Kindlin-2 reduces cell protrusion and focal adhesion formation, as well as reducing cell spreading and invasiveness/migration (40). Lower expression of Bmi-1 in miR-203 over-expressed cells can restore cell proliferation, suggesting that stem renewal factor Bmi-1 can be a direct target of miR-203, and thus regulation of Bmi-1 by miR-203 may play an important role in controlling cell proliferation and self-renewal of oesophageal cancer stem-like cells (41).

Genome-wide gene expression data and target site inhibition assays have shown that miR-203 directly regulates LIM and SH3 protein 1 (LASP1) and miR-203 can be associated with LASP1 to influence development of ESCC cells. There is also significant correlation between expression level of miR-203 and relapse-free survival (42). Tumour suppressive miR-375 can be restored by cyclic hydroxamic acid-containing peptide 31, a histone deacetylase inhibitor of ESCC cells. LDHB and AEG-1/MTDH are detected as miR-375-targeted genes, with both mRNA and protein expression levels of these verified in ESCC clinical specimens (43). In primary EC cells, miR-429 is expressed at low levels. Up-regulation of miR-429 inhibits invasion and promotes EC cell apoptosis by targeting Bcl-2 and specificity protein 1 (SP1), binding to 3'-UTRs of Bcl-2 and SP1 mRNA to reduce their expression (44). MiR-518b

can suppress cell proliferation by directly regulating Rap1b expression, leading to induction of apoptosis and repression of invasion. These data may together indicate that miR-518b functions as a tumour suppressor by targeting Rap1b in development of ESCC (45). Additionally, expression of miR-655 has been investigated in a cohort of ESCC, and its up-regulation may inhibit ESCC cell invasiveness by targeting pituitary tumour-transforming gene-1 (PTTG1) within the 3'-UTR of PTTG1 mRNA (46) (Table 1).

MicroRNAs in progression from BE to AC

The precursor BE is considered to be a main risk factor in developing EAC, as it is characterized by replacement of normal stratified squamous epithelium of the oesophagus with columnar epithelium with intestinal differentiation. Numbers of studies have examined the role of miRNAs in disease progression from BE to adenocarcinoma. When comparing different samples from BE patients with LGD, HGD and EAC, researchers have found that 9 up-regulated miRNAs, miR-126, miR-143, miR-145, miR-181a, miR-181b, miR-199a, miR-28 and miR-30a-5p, show greater expression in EAC compared to HGD, while 7 downregulated miRNAs, including miR-149, miR-203, miR-210, miR-27b, miR-513, miR-617 and miR-99a, have lower expression in EAC compared to HGD (47). Thus, miRNAs may play important roles in progression of the disease to later stages. Also, 3 oncogenic miRNAs miR-25, miR-93 and miR-106b are up-regulated in EAC relative to BE; thus there is a progressive involvement of these miRNAs in the neoplastic process (48). Progression from LGD to HGD is associated with up-regulation of miR-200a*, miR-513, miR-125b, miR-101 and miR-197, as well as down-regulation of miR-23b, miR-20b, miR-181b, miR-203, miR-193b and miR-636 (49). Moreover, transition from HGD to EAC has shown changes in 7 miRNAs, among which let-7a/b/c, miR-193a, miR-345 and miR-494 are down-regulated. Thus, some candidate miRNAs can be up-regulated or down-regulated at various stages in progression from BE to EAC. Of note, miR-192 can be up-regulated, while miR-203 and let-7 have been found to be down-regulated in multiple studies on progression from BE to EAC.

MicroRNAs as biomarkers in oesophageal cancer

Identification of miRNAs as modulators of gene expression has proposed them to be novel candidate diagnostic and prognostic indicators. MiRNAs have special characteristics, such as ease of detection and association with

established prognostic parameters, making them robust and reliable biomarkers of cancer. To use miRNAs as diagnostic tools for oesophageal cancer, different levels of expression must be found in there compared to normal or pre-malignant cells; thus specific miRNAs that are significantly up-regulated or down-regulated between these groups must be identified.

A panel of five miRNAs, including miR-15b, miR-21, miR-203, miR-486-5p and let-7a, has been used for prediction of progression of BE to EAC; it is likely that these miRNAs are implicated in development of adenocarcinoma (50). MiR-196 has also been found to increase progressively from normal squamous mucosa to LGD and then HGD to adenocarcinoma. In addition, miRNA expression profiles can differentiate between normal mucosa, squamous cell cancer, Barrett's changes, and adenocarcinoma (51). To investigate prognostic-predictive markers in EAC, some studies have revealed that miR-126 expression is correlated with tumour cell differentiation and LNM, while miR-16-2 and miR-195p are associated with LNM and higher tumour stage (52).

In ESCC, miR-34b and miR-139 are found to be consistently dysregulated, while miR-129 has been identified as a significant diagnostic factor. MiR-34b can be down-regulated in ESCC cells compared to normal ones, which is of particular interest since previous studies revealed that miR-34b could be the direct transcriptional target of p53 (53). Low-expression of miR-34b may be due to over-expression of p53 and thus miR-34b might represent a surrogate biomarker. Utility of serum miR-21 and miR-375 have been regarded as biomarkers in ESCC, as miR-21 is significantly higher, while miR-375 is remarkably lower in these patients compared to controls (54). Up-regulation of miR-25, miR-151 and miR-424 as well as down-regulation of miR-29c, miR-99a, miR-100 and miR-140* are able to predict squamous cell carcinoma compared to adjacent normal tissues (55). In ESCC, miR-200b/c, miR-205 and miR-429 are significantly higher, and their fluctuation has been validated. Thus, they can be not only biomarkers for ESCC, but could also be tumour invasion markers (56). Moreover, expressions of both miR-106a and miR-148a correlate with disease recurrence and tumour-related mortality; thereby miRNA markers might predict those at higher risk of postsurgical recurrence (57).

With high levels correlated to poor prognosis in ESCC, miR-296 aims at transcripts of MDR1 drug-resistance gene and affects sensitivity of many oesophageal cancer cells to a variety of anti-cancer drugs (58). Also, miR-27a can target MDR1 to alter oesophageal cancer cell chemo-sensitivity (59). In ESCC, low expression of miR-103 and miR-107 can be associated with good prognosis, with high miR-103 and 107 expressions as

well as LNM independently connected to poor survival (60). Over-expression of miR-92a correlates to both LNM and poor survival (61). Also, high miR-129 expression has been found to relate to poor postoperative prognosis, while low miR-145 expression is associated with poor prognosis (22).

Circulating miRNAs are promising biomarkers for non-invasive testing and dynamic monitoring in cancer patients. Serum level of miR-200c can be useful for predicting responses to chemotherapy and prognosis of patients with EC who receive neoadjuvant chemotherapy (62). Significant down-regulation of miR-107 has been observed in neoplastic and preneoplastic oesophageal tissues, suggesting that miR-107 levels may discriminate ESCC patients from healthy individuals (63). Moreover, plasma concentrations of miR-18a can be significantly higher in ESCC compared to normal; thus miR-18a may be a very useful biomarker for oesophageal cancer detection (64). With origins from plasma, circulating miR-155 has been found to have significant diagnostic value for oesophageal cancer (65) (Fig. 2a)

MicroRNAs as therapeutic targets in oesophageal cancer

Over the past year or so, there have been many experimental studies on miRNAs and EC management. MiRNAs are excellent candidates for novel molecular targeting treatments due to their ability to regulate multiple genes in molecular pathways. The reason for using miRNAs as potential anti-cancer drugs is based on two major findings: first, miRNA expression can be deregulated in cancer; second, targeting miRNA expression may change the cancer phenotype. Based on results of microarray analysis, miR-192, miR-194 and miR-622 have been selected, and expressions of these three miRNAs have been shown to be significantly reduced after neoadjuvant therapy, confirming array profiling data. Importantly, pre-therapeutic intratumoural expression of both miR-192 and miR-194 is significantly associated with histopathological response of ESCC to multimodal therapeutic treatment; thus miR-192 and miR-194 can be considered to be predictive markers for therapeutic response in multimodality therapy of patients with locally advanced EC (66). Furthermore, miR-148a transfection may result in remarkably higher sensitivity to cisplatin treatment, as it has been found to sensitize chemotherapy-sensitive EAC and ESCC cells to cisplatin and, to a lesser extent, to 5-fluorouracil and has attenuated resistance in chemotherapy-resistant variants (67). As one of the most commonly dysregulated miRNAs in many cancers, miR-21 is significantly associ-

Figure 2. MicroRNAs as biomarkers and therapeutic targets in oesophageal cancer. (a) Identification of miRNAs as modulators of gene expression renders them novel candidate diagnostic and prognostic indicators. MiRNAs have special characteristics, including stability, ease of detection and association with established clinicopathological prognostic parameters. Thus, multiple miRNAs are regarded as robust and reliable diagnostic or prognostic biomarkers in oesophageal cancer; (b) Association of aberrant miRNA expression with oesophageal cancer and functional analyses of specific miRNAs have established their key roles as tumour suppressors or oncogenes, which illustrates the feasibility of manipulating miRNA expression as a cancer therapeutic strategy.

ated with pathological stage of ESCC. The effect of miR-21 on PTEN/AKT signalling pathway has been found to be abrogated by the novel Organometallic arene Ru(II) drug Rawq01. Rawq01 can upregulate expression of PTEN through miR-21 inhibition and therefore inhibit the PI3K-AKT pathway (20). Furthermore, down-regulation of miR-27a may confer sensitivity of EC cells to drugs, which could significantly reduce expression of Bcl-2 and up-regulate expression of Bax, conferring drug-induced apoptosis by enhancing Bcl-2/Bax ratio, thereby effectively reversing drug resistance of EC cells (59).

MiRNAs can also represent effective gene therapeutic methods. Through miRNA therapy, personalized cancer treatment can either reduce activity of oncogenic miRNAs or restore levels of tumour suppressive miRNAs. Currently, there are two possible approaches for use of miRNAs as cancer therapies, either through antisense-mediated inhibition of oncogenic miRNAs or by 'replacement' of under-expressed tumour suppressive miRNAs with miRNA mimetic or viral vector-encoded miRNAs. Obvious inhibitory types of molecule to miRNA are anti-miRNA oligonucleotides (AMOs), which may prevent interactions between miRNA and target mRNAs through competition (68). Using AMOs is intended to block expression of an oncogenic miRNA, such as miR-17-29 cluster, miR-21, miR-122, miR-155 and miR-221. Moreover, the miR-mask, a sequence with perfect complementarity to the binding site for an endogenous miRNA in a target gene, can also inhibit access of endogenous miRNA to its binding site (69). Furthermore, restoring the endogenous complement of miRNAs in a cell can be achieved by either introducing a short synthetic duplex RNA molecule that is loaded

into the RISC or by inducing expression of the stem-loop pre-miRNA in a viral vector expression system or miRNA mimetics system, which mimics endogenous mature miRNA, such as let-7, miR-15 and miR-34 (70) (Fig. 2b).

However, few miRNAs can accomplish their function mainly by suppressing expression of only one or two key target genes, and thus manipulating miRNA function may be more effective than silencing one or a few oncogenes or genes required for cell proliferation or survival. In conclusion, these data show the complexity and widespread regulation of gene expression by miRNAs, which should be taken into consideration when developing miRNA-based gene therapy.

Conclusions

Understanding of regulatory roles of miRNAs has already been demonstrated in oesophageal cancer. Some miRNAs including miR-21, miR-31, miR-92a, miR-196a, miR-221/222, miR-223 and miR-577 can act as oncogenes, while others such as let-7, miR-34a, miR-98, miR-99a, miR-100, miR-129-2, miR-133a, miR-138, miR-143, miR-150, miR-200b, miR-203, miR-214, miR-375, miR-429, miR-518b, miR-655 may play roles of tumour suppressors. Thus, altered miRNA expression patterns may exert a significant impact on balance between tumour suppressors and oncogenes. Continuous expansion of identified miRNAs as biomarkers and therapeutic targets may offer fascinating insights into the complexity of gene expression regulation.

Furthermore, research into miRNA involvement in oesophageal carcinogenesis is a rewarding field, and studying altered miRNA expression patterns can offer

substantial insights into the pathogenesis of this devastating disease. Further work to identify down-stream targets of these miRNAs will improve our understanding of their mechanisms action, which will shed new light on using them clinically as biomarkers or therapeutic targets in oesophageal cancer.

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