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REVIEW ARTICLE

Potential clinical insights into microRNAs and their target genes in esophageal carcinoma

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Abstract

Esophageal carcinoma (EC) are characterized by dysregulation of microRNAs, which play an important roles as a posttranscriptional regulators in protein synthesis, and are involved in cellular processes, such as proliferation, apoptosis, and differentiation. Recently, altered miRNAs expression has been comprehensively studied in EC by high-throughput technology. Increased understanding of miRNAs target genes and their potential regulatory mechanisms have clarified the miRNAs activities and may provide exciting opportunities for cancer diagnosis and miRNA-based genetherapy. Here, we reviewed the most recently discovered miRNA target genes, with particular emphasis on the deciphering of their possible mechanisms and the potential roles in miRNAs-based tumour therapeutics.

Keywords: Esophageal tumor, microRNAs, cancer epigenetics, target gene, early diagnosis, genetherapy

Introduction

The initiation and progression of cancer, traditionally seen as a genetic disease, is now realized to involve epigenetic abnormalities along with genetic alterations (Sharma et al. 2010). The major players in epigenetic mechanisms of gene regulation are DNA methylation, histone deacetylation, chromatin remodeling, gene imprinting and small noncoding RNA expression (Banerjee and Verma 2009). At present, microRNAs (miRNAs) have become the rising stars in cancer genetics. An avalanche of research have established that miRNAs, a class of small, endogenous and non-protein-coding RNAs, provides a new tool for early clinical diagnosis (Fassan et al. 2010; Kimura et al. 2010), prognosis judgment (Hong et al. 2010; Hu et al. 2011; Hummel et al. 2010; Matsushima et al. 2010; Nguyen et al. 2010) and gene therapy (Hong et al. 2010; Mori et al. 2009; Yuan et al. 2010; Zhang et al. 2010) of Esophageal carcinoma (EC).

EC are aggressive and have poor prognosis. Although the incidence of EC has been reduced in recent decades, the five-year survival and mortality rate of EC has not significantly changed (Lv et al. 2009), and the outlook has remained bleak. Therefore, new molecular markers for detection and prognosis are urgently required. Follow-up researches on miRNAs will shed light on improving the survival rate and reducing the mortality rate of EC patients.

MiRNAs biogenesis

miRNAs genes are evolutionarily conserved, and may be located either within the introns or exons of protein-coding genes (70%) or in intergenic areas (30%) (Garofalo and Croce 2011). First, under the action of polymerase II, miRNAs are processed from primary molecules (pri-miRNAs), in which up to several kilobases are either transcribed from independent miRNA genes or are portions of introns of protein-coding RNAs (Lee et al. 2004). Pri-miRNAs then fold into hairpin structures containing imperfectly base-paired stems, catalyzed by the RNase III type endonucleases Drosha, forming pre-miRNA hairpins about 70 nucleotides (nt) long. Pre-miRNAs are transported to the cytoplasm by exportin5, where they

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are cleaved by Dicer to yield 18–22 nt miRNA duplexes. One strand is selected to function as a mature miRNA, while the other strand is degraded (Bohnsack et al. 2004; Borchert et al. 2006; Kim and Kim 2007). Mature miRNAs are combined with ribonucleoprotein complexes called miRNA-induced silencing complexes (miR-ISC), which are composed of the transactivation-responsive RNA-binding protein (TRBP), and Argonaute 2 (AGO2) (Chendrimada et al. 2005; Kim 2005). The key components of miR-ISC are proteins of the AGO family. The mature miRNA recognizes complementary sequences in the 3'-untranslated region (3' UTR) of its target mRNA, and guides the miR-ISC to repress gene expression by inhibiting translation and inducing mRNA degradation (Garzon et al. 2009; Kim 2005). MiRNAs regulate mRNAs stability and protein translation at the posttranscriptional level, and the elucidation of these mechanisms has improved our understanding of the deregulation of miRNAs in cancer. The effect of the aberrant miRNA expression on the transcriptome and proteome both accelerate tumorigenesis.

The two faces of miRNAs in tumours

Many miRNAs have a close association with cancer, in that oncogenes and tumor-suppressing genes have been found to be targets of miRNAs. MiRNAs that have oncogenic roles are categorized in oncogenic miRNAs (oncomiRs)(Harata et al. 2010; Hiyoshi et al. 2009; Kan et al. 2009; Lee et al. 2009; Tian et al. 2010; Tsuchiya et al. 2011), while those that have tumor-suppressive effects are categorized in tumor-suppressive miRNAs (ts-miRs) (Kano et al. 2010; Kitade and Akao 2010; Matsushima et al. 2010b; Yuan et al. 2010). OncomiRs that downregulate tumour suppressors (Lee et al. 2009) or other important genes involved in differentiation might contribute to tumorigenesis by stimulating proliferation (Hiyoshi et al. 2009; Lee et al. 2009; Mori et al. 2009) and invasion (Hiyoshi et al. 2009; Mori et al. 2009; Tian et al. 2010) and by preventing apoptosis (Lee et al. 2009) and increasing genetic instability (Wu 2011). A ts-miR is often downregulated or lost in a tumour. It mainly results in mutation, deletion, promoter methylation, or inhibition of the expression of a target oncogene (Kano et al. 2010; Tsuchiya et al. 2011), which subsequently contributes to tumour repression (Garzon et al. 2010). MiRNAs can act as oncogenes or tumor suppressors, depending on the type of tissues and the expression level of their targets (Garzon et al. 2009). Wu et al. showed that the expression levels of miR-143 and -145 were significantly decreased in most of esophageal squamous cell carcinoma (ESCC) tissues examined. The transfection of human EC cells with miR-143 and miR-145 expression plasmids resulted in a greater inhibition of cell mobility. Their findings suggested that the two deregulated miRNAs might act as ts-miRs of ESCC (Wu et al. 2011). Another miRNA, miR-34b has an oncogenic role in ESCC, and its expression in ESCC was significantly higher than that in the corresponding normal esophageal mucosa. It was also more highly expressed in tumors with more advanced stages, and transfection of anti-miR-34b to the ESCC cells suppressed cell growth *in vitro* (Harata et al. 2010).

Doctor Garzon hypothesized that upregulated miRNAs may act as oncogenes and downregulated miRNAs may act as tumour suppressors (Garzon et al. 2010). In addition, a recent study suggests that some miRNAs could function as oncogenes in some cell types and as suppressors in others (Garofalo and Croce 2011).

MiRNAs profiling and their target genes

Different esophageal diseases may have different expression of miRNAs. There are many associations as well as differences in their miRNAs expression profiles (Table 1). Using specified miRNAs allows differentiation among various tissue types with high accuracy. Barrett's esophageal (BE) is one of the most common premalignant

Table 1. Part of the miRNAs expression profiles in esophageal diseases.

Histologic type	Upregulated miRNAs	Downregulated miRNAs	References
BE	miR-21, miR-25a, miR-192a, miR-194a, miR-196a, miR-200c	miR-125b	Kan et al. 2009
	miR-143	miR-205	Dijckmeester et al. 2009
EAC	miR-21, miR-25a, miR-93, miR-106b, miR-192a, miR-194a, miR-196aa, miR-200c	miR-19b, miR-27b, miR-100, miR-125b, miR-203, miR-205	Kan et al. 2009
	miR-223, miR-375		Mathe et al. 2009
		miR-143, miR-145, miR-215	Wijnhoven et al. 2010
ESCC	miR-9, miR-15b, miR-16, miR-17-5p, miR-20a, miR-20b, miR-21, miR-25, miR-34b, miR-34c, miR-107, miR-127, miR-129, miR-130a, miR-130b, miR-132, miR-134, miR-137, miR-138	miR-133a, miR-133b, miR-139, miR-145	Ogawa et al. 2009
	miR-151, miR-424	miR-29c, miR-99a, miR-100, miR-140	Guo Y et al. 2008
	miR-100, miR-146, miR-155, miR-220, miR-296, miR-483, miR-494	miR-143, miR-339	Hong et al. 2010
	miR-10b		Tian et al. 2010
	miR-342	miR-203, miR-205	Feber et al. 2008
	miR-373		Lee et al. 2009
		miR-375	Mathe et al. 2009

lesions and can sometimes progress to esophageal adenocarcinoma (EAC). Dijckmeester et al. measured miRNA expression levels in neosquamous and normal squamous mucosa samples, which had been ablated by argon plasma coagulation (APC) from nine BE patients. miR-143 expression was elevated in both neosquamous and the squamous mucosa above the metaplastic segment, suggesting that miR-143 could promote a Barrett's epithelium gene expression pattern and might have a role in development of BE (Dijckmeester et al. 2009). VanBaal et al. also demonstrated specific miRNAs characteristics for normal epithelia (NE), BE and EAC. In their delicate work, miR-192, 194, 143 and 145 were highly expressed in EAC and BE compared to NSE. MiR-203 and 205 were highly expressed in NE compared to EAC and BE. In addition, miR-214 was highly expressed in EAC compared to BE and NE. Strikingly, miR-215 and 199a-5p were the most informative with EAC:BE:NE ratios of 18:37:1 and 9:4:1, respectively (Van Baal et al. 2009). Another report suggested that miR-194 might contribute to the intestinal differentiation observed in BE, and also contributes to the molecular phenotype required for tumor metastasis, miR-203 expression is lost in BE, and therefore it is likely that the miR-203-directed mechanism of epithelial replacement is lost in this tissue (Smith et al. 2010). Wijnhoven et al. also found that expression of miR-143, miR-145, and miR-215 was lower in EAC than in BE (Wijnhoven et al. 2010). The researchers in the Anderson Cancer Center used unsupervised hierarchical clustering and class comparison analyses to detect the expression levels of miRNAs in BE, low-grade intraepithelial neoplasia (LGIN), high-grade intraepithelial neoplasia (HGIN) and EAC. They confirmed that special miRNAs propiles could differentiate the EAC from NSE with almost 100% accuracy (Yang et al. 2009). It is generally accepted that EAC develops through progression from BE, as a premalignant condition: the dysplasia of BE is the most predictive marker for the risk of EAC, and the degree of BE's dysplasia has positive association with risk of EAC (Tischoff and Tannapfel 2008). The incidence of Barrett's esophgeal adenocarcinoma has been increasing at an alarming rate in Western countries. Follow-up research in this field will be expected to discriminate the subtype of BE patients who might be at high risk of progression to EAC.

MiRNAs can also be correlated with different clinicopathological classifications, especially in ESCC. Inoue et al. showed that MiR-10a's expression level was comparably downregulated in the tumors of HGIN and non-invasive ESCC, while it was elevated in the invasive ESCC tumors. They subsequently detected expression levels of miR-10a in malignant (OE21 and TE10) and non-malignant (Het1A) ESCC cell lines, and found it was significantly downregulated in malignant ESCC cell lines (Inoue et al. 2010). Mathe et al. studied miRNAs' expression in ESCC and EAC, and found that in EAC patients, the expression levels of miR-21, miR-223, miR-192, and miR-194 were elevated. By contrast miR-203, which can inhibits proliferation and invasion of ESCC, showed reduced expression in cancerous compared with noncancerous tissue. This elegant experiment showed that the expression levels of miR-21 were elevated in both noncancerous and cancerous tissue of ESCC patients, miR-375 was reduced in ESCC patients, but elevated in EAC patients, and neither miR-21 nor miR-375 associated with administration of neoadjuvant chemoradiation therapy (Mathe et al. 2009). Feber et al. also confirmed that miR-203 and miR-205 were expressed 2–10 fold lower in ESCC and EAC compared with NSE. In addition, miR-21 expression was 3–5-fold higher in both tumors versus NSE, miR-194, miR-192 and miR-200c were significantly upregulated in EAC, while compared with NSE, miR-342 was differentially expressed in ESCC, prediction analysis of microarray (PAM) was able to separate the three main types of samples into distinct groups; only BE samples were classified incorrectly (Feber et al. 2008). Matsushima et al. also found that miR-205 and miR-10a were significantly altered in cellular expression, and might be specific for ESCC, with potential roles in ESCC pathogenesis (Matsushima et al. 2010b). Taken together, these exploratory analyses highly suggested that the miRNAs might possess physiological significance in the regulation of EC development.

Several independent studies have demonstrated that the target genes of these dysregulated miRNAs may be expected to be oncogenes or tumor-suppressor genes. We generalized the main kinds of miRNA target genes and their potential roles in EC (Table 2). Taking miRNA-196a as an example, ANXA1 is considered to mediate proliferation and suppress apoptosis, and miRNA-196a showed significant inverse correlation with ANXA1 mRNA levels in esophageal cancer cell lines. This confirmed that miR-196a directly targets ANXA1 3'-UTR, thereby exerting antiapoptotic effects and contributing to EAC cell survival (Kan et al. 2009; Luthra et al. 2008). Maru et al. also ascertained that miR-196a specifically targets the 3'-UTRs of KRT5, SPRR2C and S100A9 mRNA, reducing the expression of these proteins (Maru et al. 2009). LATS2 is a member of the LATS tumor-suppressor family, which can regulate the cell cycle and apoptosis. MiR-373 as an oncomiRs that participates in ET's carcinogenesis by directly suppressing LATS2 expression, manipulating the expression of miR-373 can affect ET cells growth (Lee et al. 2009). KLF4, another known tumor-suppressor gene, has been reported to suppress ESCC cell migration and invasion, and is a direct target of miR-10b. Overexpression of miR-10b in human ESCC cells lines led to a reduction of endogenous KLF4 protein, while silencing of miR-10b caused upregulation of the KLF4 protein. And rescue experiment could partially abrogate the effect of miR-10b on cell migration and invasion (Tian et al. 2010). It also has been shown that the miR-106b-25 polycistron affects proliferation, antiapoptosis, and cell cycle-promotion in EC cells and has a role in tumorigenic activity in NE, BE, and EAC tissues. It has since been demonstrated that miRs-93 and-106b targeted

Table 2. MiRNAs and their reported target genes in esophagus.

MiRNA	Role	Species	Target gene	Functions	Detection method
miR-10b	oncomiR	ESCC cell lines	KLF4	Cell migration, cell invasion	qRT-PCR
miR-21	oncomiR	BE, EAC & EAC cell lines	PDCD4	Cell cycle, cell proliferation, cell apoptosis	Microarray, qRT-PCR
miR-25*	oncomiR	BE, EAC & EAC cell lines	Bim	Apoptosis	Microarray, qRT-PCR
miR-93*	oncomiR	EAC & EAC cell lines	P21	Cell cycle	Microarray, qRT-PCR
miR-106b*	oncomiR	EAC & EAC cell lines	P21	Cell cycle	Microarray, qRT-PCR
miR-133a/b#	ts-miR	ESCC & ESCC cell lines	FSCN1	Repress cell proliferation & invasion	Microarray, qRT-PCR
miR-145#	ts-miR	ESCC & ESCC cell lines	FSCN1	repress cell proliferation & invasion	Microarray, qRT-PCR
miR-196a	oncomiR	BE, EAC & EAC cell lines	ANXA1	Repress tumor suppressor, neoplastic progression	qRT-PCR
	oncomiR	BE, EAC & EAC cell lines	S100A9, SPRR2C, KRT5,	Neoplastic progression	qRT-PCR
miR-200	oncomiR	BE, EAC & EAC cell lines	ZEB1, ZEB2,	Induce EMT	qRT-PCR
	oncomiR	ESCC & ESCC cell lines	Delta Np63	Inhibit cell proliferation	qRT-PCR
miR-205	ts-miR	EAC & EAC cell lines, ESCC & ESCC cell lines	ZEB1, ZEB2	Repress EMT& cell proliferation	qRT-PCR
miR-210	ts-miR	ESCC & ESCC cell lines	FGFRL1	Induce cell death & cell-cycle arrest, epress cell proliferation	qRT-PCR
miR-373	oncomiR	ESCC & ESCC cell lines	LATS2	Repress tumor suppressor, cell proliferation	qRT-PCR, Microarray

All information came from following references (in order): (Tian et al. 2010; Hiyoshi et al. 2009; Kan et al. 2009*; Kano et al. 2010#; Luthra et al. 2008; Maru et al. 2009; Ohashi et al. 2009; Yuan et al.2011; Matsushima et al. 2010b; Tsuchiya et al. 2011; Lee et al. 2009).

and inhibited p21 by mRNA degradation, whereas miR-25 targets and inhibits Bim by translational inhibition (Kan and Meltzer 2009; Kan et al. 2009), resulting the formation and proliferation of EC. Recently, Ohashi et al. determined the role of microRNA-200 family members in miRNA-mediated posttranscriptional regulation of ZEB1/2, through Notch signaling, which contributes to ET initiation and progression (Ohashi et al. 2009). In Matsushima's study, expressions of miR-205 and miR-10a were increased and decreased, respectively, in neoplastic and non-neoplastic samples of ESCC patients. Alteration of miR-205 expression could modulate the phenotype of epithelial cells towards epithelial mesenchymal transition (EMT), characterized by reduced abundance of E-cadherin, which is the ESCC-specific miRNA target, and inhibition of the E-cadherin repressors, ZEB1 and ZEB2. However, miR-10a, as a tumor-suppressor, could control cell migration and invasion by targeting homeobox genes (Matsushima et al. 2010a; Matsushima et al. 2010b). FSCN1 is associated with ESCC carcinogenesis by inhibiting cell growth and invasion. Kano et al. first showed that miR-145, miR-133a, and miR-133b inhibited cell proliferation and cell invasion in ESCC cells, and that these miRNAs had conserved sequences in the 3'UTR of FSCN1. They subsequently demonstrated that a combination of these miRNAs inhibited FSCN1's expression (Kano et al. 2010). Takashima et al. showed that downregulation of PABPC1 was associated with tumor progression, including increased tumor size, locally invasive tumors, and poor overall survival (Takashima et al. 2006). Separately, Yoshino et al. confirmed that overexpression of FGFR2 was highly correlated with well-differentiated of EC (Yoshino et al. 2007). As two of the predicted targets for mir423, PABPC1 and FGFR2 were shown to be associated with the etiology or prognosis of EC (Ye et al. 2008). Tsuchiya et al. also found that miR-210 is downregulated in human ESCC and ESCC cell lines, which inhibits cancer cell survival and proliferation by inducing cell death and cell-cycle arrest in G1/G0 and G2/M. They also identified fibroblast growth factor receptor-like 1 (FGFRL1) as a target of miR-210 in ESCC, which accelerates cancer cell proliferation by preventing cell- cycle arrest in G1/G0 (Tsuchiya et al. 2011).

MiRNAs and its clinical insights

Aberrant miRNA expression is now recognized as a common feature of cancers, and specific miRNAs are being considered as potential new biomarkers for cancer diagnosis and prognosis. Fassan et al. confirmed that specific miRNAs involved in BE carcinogenesis might represent a novel diagnostic and prognostic tool in the characterization of Barrett's adenocarcinoma gene

targets (Fassan et al. 2010). In the experiment of Maru et al. the progression of NSE-BE-LGIN-HGIN-EAC was associated with a concomitant increase of miR-196a levels (Maru et al. 2009). MiR-196a may also constitute a good biomarker of progression during BE–EAC carcinogenesis (Kan and Meltzer 2009; Maru et al. 2009). Mathe's research also suggested that the reduced levels of miR-375 in cancerous tissue of EAC patients with Barrett's esophgus were strongly associated with worse prognosis (Mathe et al. 2009). In ESCC, Matsushima et al. showed that miR-205, and members of named miR-200 family, miR-200b, -200c, and -429, were significantly increased in both ESCC cell lines compared to Het1A, while nine miRNAs were significantly decreased. They confirmed that fluctuation of these miRs could be not only biomarkers for ESCC, but could also be tumor invasion markers (Matsushima et al. 2010a). Hummel et al. confirmed that the expression alterations of miR-21 correlate with tumor location and lymph node status in patients with locally advanced ESCC, and demonstrated that miR-106a and miR-148a expression correlates with disease recurrence and tumor-related mortality. Therefore, miRNA markers might inform the initial assessment of these patients and predict those at higher risk of postsurgical recurrence (Hummel et al. 2010). Guo et al. also showed that high expression of hsa-miR-103/107 correlated with poor survival, by univariate analysis as well as multivariate analysis (Guo et al. 2008). In addition, EC with low GNG7 expression invaded deeper than those with high GNG7 expression, while GNG7 expression was significantly associated with the presence of miR-328 in esophageal cancer cell lines. This suggests that miR-328 might be a regulator of GNG7 expression, and GNG7 suppression represents a new prognostic indicator of ET (Ohta et al. 2008). Recently, Hong et al. indicated that low expression of miR-296 was able to distinguish long-term survivors with node-positive disease from those dying within 20 months by predicting survival (Hong et al. 2010). In Japan, Ogawa et al. detected the expression of miRNAs in 30 primary ESCC specimens by qRT-PCR. The expression levels of miR-34b were considerably higher in the tumor tissue, but there was little or no expression in NSE. They also observed that the overexpression of miR-129 was a significant and independent prognostic factor in surgically treated ESCC patients (Ogawa et al. 2009).

In addition, detecting the expressions of miRNA-related genes could also monitor the prognosis of ET. Exportin 5(XPO5) mediates pre-miRNA nuclear export and Melo et al. demonstrated the presence of XPO5-inactivating mutations in a subset of human tumors with microsatellite instability. The XPO5 genetic defect traps pre-miRNAs in the nucleus, reduces miRNA processing, and diminishes miRNA target inhibition. The restoration of XPO5 functions not only reversed the impaired export of pre-miRNAs but also displayed tumor-suppressor features (Melo et al. 2010). RNASEN is thought to participate in processing of pri-miRNA and essential for miRNA processing. And the level of RNASEN were elevated in ESCC and its expression correlated with poor ESCC prognosis (Guo et al. 2008; Ogawa et al. 2009).

Furthermore, single nucleotide polymorphisms single nucleotide polymorphisms (SNPs) in miRNA-related genes may increase the risk of ET. For example, SNPrs6505162, which is located in the pre-mir423 region, was associated with a significantly reduced risk, a polymorphism in the pre-mir196a-2 gene was associated with 1.7-fold increased risk, and a polymorphism in the pre-mir631 gene was associated with a significantly increased risk of ET (Ye et al. 2008). In addition, SNPrs2910164 is located in the sequence of the miR-146a precursor. Recently, Guo et al. found there was a strong correlation between the rs2910164 C/G variant and the clinical TNM stage in ESCC. Their findings suggest that this functional SNP in pre-miR-146a could contribute to ESCC susceptibility (Guo et al. 2008). Harris and others also have identified that inflammatory risk score (IRS) and miR-21 expression are independent predictors of prognosis, and together may be clinically useful in identifying patients with early stage cancer at high risk of metastases (Harris 2010). Recently, Merritt et al. reported that decreased expression of key RNAi enzymes, Dicer and Drosha, in epithelial ovarian cancers was associated with poor clinical outcome in patients (Merritt et al. 2010).

Accordingly, both miRNAs and miRNA-related genes have a close relationship with EC. After continuous study, miRNAs may become good screening biomakers for prognosis of BE and for the early stage of EC with high sensitivity and specificity.

MiRNAs and gene therapy

Traditional treatments of tumours mainly comprise surgical treatment, chemotherapy, radiotherapy, and immunotherapy. However, only few drugs are available that can be used safely and effectively for cancer therapy. The molecular heterogeneity, which leads to different individual effects, is the main barrier to effective treatment. To improve this situation, the development of novel individual and highly specific targets for cancer therapy is of the utmost importance.

MiRNAs are excellent candidates for novel molecular targeting treatments because of their ability to regulate multiple genes in molecular pathways. The reason for using miRNAs as a potential anticancer drugs is based on two major findings: first, miRNA expression is deregulated in cancer compared with normal tissues; second, the cancer phenotype can be changed by targeting miRNA expression. Dr. Garzon conceived two main strategies to target miRNA expression in cancer: first, direct strategies that involve the use of oligonucleotides or virus-based constructs to either block the expression of an oncomiRs or to substitute for the loss of expression of ts-miRs; and second indirect strategies that involve the use of drugs to modulate miRNA expression by targeting their transcription and their processing (Garzon et al. 2010).

There are a number of ways to block oncomiRs, such as antisense oligonucleotides, miR-masks, miRNA sponges, and small RNA inhibitors. Antisense oligonucleotides can

bind to target miRNAs following the Watson–Crick complementarity and induce either degradation or duplex formation. However, the miR-mask is a kind of synthetic oligonucleotide that competes with endogenous miRNAs for the same target mRNA by complementary binding to the 3'UTR of their targets. MiRNA sponges are oligonucleotide constructs with multiple complementary miRNA binding sites to target miRNAs. These sponges will 'soak up' endogenous miRNAs, decreasing the expression levels of oncomiRs. Small RNA inhibitors bind sequence-specifically to miRNA and effective inhibit the miRNA. To substitute for the loss of expression of ts-miRs, miRNA mimics could mainly be used. These synthetic miRNAs could restore the downregulated miRNA expression. Another way to restore the downregulated miRNAs expression is to insert genes coding for miRNAs into viral constructs, such as the adeno-associated virus (AAV) (Garzon et al. 2010).

In recent years, outstanding contribution have been made to the development of miRNAs-based tumour treatments, aided by the discovery of numerous miRNA targets and related regulation-pathways. A small group of miRNAs, including miR-155, let-7a, miR-21, and the miR-17-92 cluster, are aberrantly expressed in a wide variety of hematological malignancies and solid tumors. Therefore, developing a strategy to silence or restore the expression of these oncomiRs would have an impact on multiple groups of cancer patients (Garzon et al. 2009). Kota et al. demonstrated that expression of miR-26a, which induces cell-cycle arrest by direct targeting of cyclins D2 and E2, is reduced in hepatocellular carcinoma cells. Systemic administration of miR-26a in mouse model of liver cancer using AAV results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and dramatic protection from disease progression, without toxicity (Kota et al. 2009). These findings suggest that delivery of miRNAs that are highly expressed and therefore tolerated in normal tissues, but lost in disease cells, may provide a general strategy for miRNA replacement therapies.

MiR-21 is one of the most commonly deregulated miRNAs in many malignancies: its expression level is also significantly associated with pathological stage in ESCC (Mori et al. 2009). Patients with lymph node metastasis or venous invasion showed significantly high expression of miRNA-21 in seven ESCC cell lines. Meanwhile, anti-miRNA-21 transfected cells showed significant reduction in cellular proliferation and invasion. In addition, inhibition of miRNA-21 increased sensitivity to anticancer drugs in cancer cell lines; Thus, chemotherapy combined with miRNA-21 suppression may be more effective than chemotherapy alone (Hiyoshi et al. 2009). Zhang et al. identified that miR-27a might be a regulator of multidrug resistance gene 1 (MDR1). Downregulation of miR-27a could confer sensitivity of both P-glycoprotein (P-gp) related and P-gp non-related drugs on EC cells, and might promote ADR-induced apoptosis, accompanied by increased accumulation and decreased releasing amount of ADR. This would significantly decrease the expression of P-gp, Bcl-2, and the transcription of the MDR1, but up-regulate the expression of Bax (Zhang et al. 2010). These findings verified that anti-miRNAs oligonucleotide medicines have potential value in clinical treatment and serve as a novel therapeutic target in ESCC. In China, the most common pathological type of EC is ESCC. Accordingly, these studies have very important and realistic significance.

Although this body of research provides encouraging support for the potential of miRNA-based cancer therapy, there are much problems in molecule biostability, specificity and delivery in the development of miRNA-based gene therapy. Locked nucleic acid (LNA) are novel, third-generation RNA analogs that display most of the characteristics required to make potent and safe antisense drugs (Cho 2010). MiR-122 is a liver-expressed miRNA implicated in cholesterol and lipid metabolism and in hepatitis C virus (HCV) replication. Elmen et al. confirmed that acute administration by intravenous injection of LNA-antimiR to African green monkeys was accompanied by depletion of mature miR-122 and dose-dependent lowering of plasma cholesterol, without any toxicities or histopathological changes in the study animals (Elmen et al. 2008). After this a phase I clinical trial of the world's first miRNA drug (LNA-antimiR-122) has been launched, confirming the use of antagomirs to inhibit the activity of specific miRNAs as a possible approach for miRNA medicine.

Few of miRNAs may accomplish their function mainly by suppressing the expression of only one or two key target genes, and Dr. Petrocca has indicated that it is not clear whether manipulating miRNA function will be more effective than silencing one or a few oncogenes or genes required for cell proliferation or survival. He considered that only a direct comparison of silencing a few key oncogenes with inhibiting or activating an oncomiR in animal models will help answer this question (Petrocca and Lieberman 2009). Apart from this, recent studies have reported that miRNAs can also regulate gene expression at the transcriptional level by binding directly to the DNA (Garzon et al. 2010).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.

Prospects

The abnormal function of miRNAs could be caused by several mechanisms, including genomic deletion, mutation, epigenetic silencing, and/or miRNA processing alterations (Garzon et al. 2009). DNA hypermethylation in the miRNA 5'regulatory region is a mechanism that can account for the downregulation of miRNA in tumors (Esteller 2008). The abnormal expression and function of miRNAs may induce different kinds of tumours. However, using miRNA mimics and antisense RNA techniques could rectify abnormal changes in miRNAs, normalizing the gene regulatory networks and signaling pathways and reverse the phenotype of cancer cells.

The mechanism of the occurrence of EC remains unclear: cell malignant transformation might be influenced by genetic background and environmental factors, such as hereditary factors, smoking and drinking, diet habit, and chronic esophageal injury. BE is the most common precancerous lesion and often advances to EAC. Although endoscopic and histological diagnosis are still the gold standard for surveillance of patients with BE, both are limited, either by sampling errors in biopsies or by differences in histological interpretation (Tischoff and Tannapfel 2008). Thus, it is necessary to identify new biomarkers of EC. MiRNAs and related genes have been demonstrated to be potent tools for the diagnosis and treatment of EC in the future. However, there are still much obstacles to be overcome before clinical trails of miRNA drugs in cancer treatment can commence, such as the delivery of miRNAs to a specific tissue or disease site, avoiding off-target effects, optimizing the dose, assessing possible toxic reaction and minimizing the likelihood of immune activation (Garzon et al. 2010; Wang and Wu 2009). At present, systemic delivery usually uses liposomes and viral vectors; however, nanoparticles might offer new opportunities in this field (Wang et al. 2010). Ongoing progress in the miRNA field will bring a better understanding of miRNA biogenesis and function, which will certainly affect the development of miRNA-based gene therapy.

Declaration of interest

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