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REVIEW

MiRNAs in oesophageal squamous cancer

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ABSTRACT

Oesophageal cancer is a common cancer worldwide with a very poor prognosis. Oesophageal squamous cell carcinoma (OSCC) is the major subtype of oesophageal cancer but also one of the least studied cancers worldwide. Although the molecular genetics of OSCC have been widely studied, the molecular mechanism of OSCC carcinogenesis is not completely understood. MicroRNA (miRNA) is now essential to understanding the molecular mechanism of cancer progression. Recent findings include the following: 1) recent findings regarding the functions of miRNA; 2) some of the latest findings on expression profile of miRNA involved in OSCC; 3) miRNAs and their target genes and molecular mechanisms in OSCC; and 4) the therapeutic-clinical potential of miRNAs in OSCC. We can make full use of this knowledge to guide us to evaluate and improve the patient's condition and choose the most fitting medical treatment or explore new approaches to improve the survival ratio of OSCC patients.

KEYWORDS

MicroRNA, oesophageal cancer, oesophageal squamous cell carcinoma

INTRODUCTION

According to statistics, oesophageal cancer, which occurs worldwide with a variable geographic distribution, is the sixth leading cause of death from cancer and one of the least studied cancers worldwide.[1,2] It has two main forms, each with distinct aetiological and pathological characteristics: oesophageal squamous cell carcinoma (OSCC) and oesophageal adenocarcinoma (OAC). OSCC is the most frequent subtype of oesophageal cancer; it is often diagnosed at an advanced stage and its prognosis remains poor. Although preoperative chemotherapy and chemoradiation therapy are currently used for patients with advanced stage OSCC, their effectiveness is unsatisfactory. Consequently, it is important for targeted prevention and early detection in the control of this disease. MicroRNAs (miRNAs) are a species of small noncoding single-stranded RNA of about 19 to 24 nucleotides that through partial sequence homology may interact with the 3′-untranslated region of target mRNA molecules. MiRNA inactivates multiple target genes by sequence-specific binding-mediated destabilisation of mRNA or inhibition of translation.

In this review, we highlight four issues of current relevance to this field and discuss 1) the recent findings regarding the functions of miRNA; 2) some of the latest findings on molecular mechanisms of miRNA involved in OSCC; 3) miRNAs and their target genes in OSCC; and 4) the therapeutic-clinical potential of miRNAs in OSCC.

PREVALENCE AND AETIOLOGY OF OESOPHAGEAL CANCER AND OESOPHAGEAL SQUAMOUS CANCER

According to statistics, oesophageal cancer is the sixth most common cancer worldwide and the third malignancy of the gastrointestinal tract with a very poor prognosis.[3] Oesophageal cancer has two major histological types: adenocarcinoma (OAC) and squamous cell carcinoma (OSCC); OAC predominantly occurs in Western societies, but OSCC is the predominant histological type of oesophageal cancer worldwide.[4] Incidence rates vary across the world by nearly 16-fold, with the highest rates found in Southern and Eastern Africa and Eastern Asia, and lowest rates observed in Western and Middle Africa and Central America in both males and females.[3] OSCC is also one of the least studied cancers worldwide but one of

the most lethal malignancies, with a five-year survival rate of less than 10%, and occurs at a relatively high frequency in certain areas of China.[5,6] Thus, OSCC is a very deadly disease in strong need of new, effective, therapeutic approaches.

Major risk factors for OSCC are not well understood, but are thought to include tobacco smoking, alcohol consumption, poor nutritional status and low intake of fruits and vegetables.[7-11] In some low-risk areas such as the United States and some Western countries, cigarette smoking and excessive alcohol consumption account for about 90% of the total cases of OSCC.[12] But the rates in these areas have been steadily declining because of long-term reductions in smoking and alcohol consumption.[13] However, OSCC has been increasing in certain Asian countries, probably as a result of increases in tobacco use and alcohol consumption.[14] The distinct risks exhibited by individuals exposed to similar known risk factors implied that genetic predisposition might play an important role in oesophageal cancer aetiology.[7,8] The exact causes and the mechanism of OSCC, which appear to be multifactorial, have not yet been fully appreciated. Thereby there is no effective strategy for treatment and prevention nowadays.

OSCC is often not diagnosed until a late stage when it is incurable and the prognosis of affected patients is unsatisfactory. Consequently, the dismal outcome of oesophageal cancer patients highlights the need for novel prognostic biomarkers to allow a tailored multimodality approach with increased efficacy, such as microRNAs (miRNA).

MiRNAs AND THEIR FUNCTIONS

MicroRNAs are small noncoding RNAs of 19-24 nucleotides in length that are important in the regulation of several crucial biological processes, such as cell growth,[15] apoptosis,[16] development,[17] differentiation,[18] and endocrine homeostasis.[19] The first miRNA was described in 1993, in which the C. elegans heterochronic gene lin-4 encoded small RNAs with antisense complementarity to lin-14.[20] MiRNAs play important roles in the aetiology of many human diseases by post-transcriptionally regulating the expression of approximately one third of all human genes.[21,22] It has been investigated in a variety of diseases, including diabetes, heart diseases, Alzheimer's disease, and viral infections.[23] The most active area and the starting point for the pathogenetic role of miRNAs lies in cancer research.[24,25] MicroRNAs are known as gene silencers and their expression profiles have been reported to be negatively correlated with those of their target genes.[26] The biological role of miRNA is to inactivate single or multiple target genes by sequence-specific binding-mediated destabilisation of mRNA or inhibition of translation mechanisms.[27] The degradation or inhibition of specific mRNA translation could decrease the expression of the resulting protein, and their role in disease development would presumably be through the regulation of their target protein gene.[28,29] Recently, genome-wide expression of miRNAs, which could be examined by microarray and on a more limited miRNA set by microbead hybridisation or reverse transcription-PCR (RT-PCR), can provide increasing information in miRNA function studies of many diseases.

MiRNAs AND CANCERS

Growing evidence has indicated important roles for different miRNA species in the development of different cancers, perhaps being involved in the pathophysiology of all human cancers.[30] MiRNA expression profiles have frequently been reported to be correlated with the aetiology, classification, progression, and prognosis of multiple human cancers.[1,21,29,31-33] Recent studies indicate that numerous miRNAs have been identified as tumour-related and can be categorised in two groups based on their functional relevance, tumour suppressors and oncogenes, and the miRNAs with roles in cancer are designated as oncogenic miRNAs (oncomiRs).[34] MiRNAs can act as oncogenes or tumour suppressors and modulate the expression of approximately one-third of all human genes.[35] In normal cells, tumour suppressor miRs are highly expressed and downregulate the expression of oncogenic proteins, whereas in tumour cells they are silenced leading to upregulation of oncogenic proteins. Conversely, oncomiRs are upregulated in tumour cells, downregulating the expression of tumour suppressive proteins. Both tumour suppressor miRs and oncomiRs are involved in biological and pathological processes including cell differentiation, proliferation, apoptosis and metabolism, and are emerging as highly tissue-specific biomarkers with potential clinical applicability for defining cancer types and origins.[36]

MiRNAs IN OSCC

In recent years, the study of miRNAs in OSCC has been expanded and is producing new knowledge on the molecular basis of this disease. MicroRNA expression profiles are important diagnostic and prognostic markers of OSCC. We summarised the miRNAs that have been reported in recent years[1,6,32,37-66] (*table 1*) and discovered that more upregulated miRNAs species than downregulated species have been found. In other words, this means that we have found more oncomiRs than tumour suppressors

Table 1. *MiRNAs in oesophageal squamous cell carcinoma that have been reported in the literature*

	Upregulation			Downregulation	
Tissue	miR-9	miR-10b	miR-15b	let-7	
	miR-16	miR-17-5p	miR-20a	miR-29c	miR-30a-3p
	miR-20b	miR-21	miR-23a	miR-100	miR-106a
	miR-25	miR-26a	miR-27b	miR-125b	miR-133a
	miR-31	miR-34b	miR-34c	miR-133b	miR-139
	miR-92a	miR-93	miR-96	miR-143	miR-145
	miR-103	miR-106a	miR-107	miR-148a	miR-203
	miR-127	miR-128b	miR-129	miR-205	miR-210
	miR-130a	miR-130b	miR-132	miR-375	
	miR-134	miR-137	miR-138		
	miR-142-3p	miR-151	miR-192		
	miR-194	miR-205	MiR-223		
	miR-296	miR-373	miR-1322		
Cell	miR-16	miR-21		miR-10a	miR-29c
	miR-30a-5p	miR-141		miR-99a	miR-100
	miR-200c	miR-205		miR-125b	miR-146b
	miR-429			miR-153	miR-210
				miR-376a	miR-379
				miR-593	miR-651

correlated with OSCC. Furthermore, increasingly relevant research is being performed. For example, some studies have revealed that functional single nucleotide polymorphisms (SNPs) rs2910164 in pre-miR-146a and rs11614913 in pre-miRNA-196a could contribute to OSCC susceptibility and clinical outcome in Chinese Han.[67,68] Zhang *et al.*[58] identified a profile of seven serum miRNAs (miR-10a, miR-22, miR-100, miR-148b, miR-223, miR-133a, and miR-127-3p) as OSCC biomarkers. And Chen *et al.*[69] concluded that CpG island methylation of miR-34a, miR-34b/c and miR-129-2 occurs frequently and is an important mechanism, for their low expression in OSCC and the high methylation ratio of miR-129-2 indicated its potential as a methylation biomarker in the early diagnosis of OSCC.

MiRNAs AND THEIR TARGET GENES IN OSCC

Altered miRNA expression has been found to promote carcinogenesis, but little is known about the role of miRNAs in oesophageal cancer. Under these circumstances, accumulating research concerns the target genes of specific miRNAs in OSCC to elucidate the exact mechanism of OSCC carcinogenesis. The target genes of these causal miRNAs may be tumour suppressor genes or other genes related to oncogenes, such as growth factors, growth factor receptors, signal transducers, transcription factors, programmed cell death regulators, genes that control cell division, or genes that repair DNA.[1]

OncomiRs are presumed to function by downregulating tumour suppressor genes. Several reports in the literature have mentioned the target genes of several oncomiRs in OSCC (*table 2*). Many studies described that miR-21 targets phosphatase and tensin homolog (PTEN),[70] tumour suppressor gene tropomyosin 1 (TPM1),[71] programmed cell death 4(PDCD4),[43,72] Sprouty2,[73] B-cell CLL/lymphoma 2(BCL2), programmed cell and Maspin,[70,74-76], thereby demonstrating its involvement in tumour growth, invasion, and metastasis.[50] Zhang *et al.*[63] revealed that miR-31 promoted OSCC colony formation, migration and invasion that may regulate three tumour suppressor genes, namely epithelial membrane protein 1 (EMP1), kinase suppressor of ras 2 (KSR2) and regulator of G-protein signalling 4

Table 2. *MiRNAs and their target genes in oesophageal squamous cell carcinoma*

	MiRNAs	Target gene	Effect of OSCC
OncomiRs	miR-10b	KLF4	Promotes cell migration and invasion
	miR-19a	TNF-α	Promotes cell growth
	miR-21	PTEN, TPM1, PDCD4, Sprouty2, BCL2, maspin	Promotes cell growth, invasion, and metastasis
	miR-31	EMP1, KSR2, RGS4	Promotes tumour colony formation, migration and invasion
	miR-34b	p53	DNA damage and oncogenic stress
	miR-92a	CDH1	Promotes cell migration and invasion
	miR-205	ZEB2	Promotes cell invasion and migration
	miR-373	LATS2	Affects cell cycle progression or apoptosis
	miR-296	Cyclin D1, p27	Affects tumour cell growth
Tumour suppressor	let-7	HMGA2	Inhibits cell proliferation, lymph node metastasis
	miR-29c	Cyclin-E	Inhibits tumour cell growth
	miR-133a/b	FSCN1	Inhibits cell proliferation and invasion
	miR-145	FSCN1	Inhibits cell proliferation and invasion
	miR-203	ΔNp63	Inhibits cell proliferation
	miR-210	FGFRL1	Inhibits cell proliferation

(RGS4). Hong *et al.*[55] found that miR-296 might cause the growth of OSCC *in vitro* and *in vivo* through regulation of cyclin D1 and p27. He *et al.*[40] demonstrated that miR-34b was the direct transcriptional target of protein 53 (p53) in human and mouse cells and that its induction by DNA damage and oncogenic stress was p53 dependent. Matsushima *et al.*[51] showed that miR-205 was likely to control cell invasion and migration in OSCC cells through its repression of zinc finger E-box-binding homeobox 2 (ZEB2), a repressor of E-cadherin expression resulting in dysregulating cellular processes which may ultimately lead to tumourigenesis of OSCC. Tian *et al.*[42] described that miR-10b overexpression promoted cell migration and invasion in human OSCC cell lines by regulating the Krüppel-like factor 4 (KLF4), a zinc finger protein of the Krüppel-like factor family that plays a role in cell cycle regulation, differentiation, and rises in response to DNA damage.[77, 78] Lee *et al.*[49] described that the miR-373 expression was found to be inversely correlated with large tumour suppressor homolog 2 (LATS2) expression in the OSCC cell lines and OSCC patients. After detecting 109 consecutive OSCC samples, Kurashige *et al.*[66] found that high expression of miR-223 had a significant adverse impact on the survival of OSCC patients through repression of the function of FBXW7.

Conversely, it can be postulated that re-introduction of tumour suppressor miRs into tumour cells will result in upregulating tumour suppressor genes leading to the downregulation of target oncogenes and tumour suppression (*table 2*). Liu *et al.*[59] demonstrated that let-7, a tumour suppressor, was expressed lower in OSCC and might repress cell proliferation, and correlated with lymph node metastasis of OSCC patients by negatively regulating high mobility group AT-hook 2 (HMGA2) at the post-transcriptional level. Ding *et al.*[6] reported that miR-29c, a potential tumour-suppressing miRNA in OSCC development, could influence the activity of cyclin E-CDK2 complexes by inhibiting the expression of cyclin E, one of the human G1 cyclins that binds to and activates its catalytic partner cyclin-dependent kinase 2 (CDK2) which phosphorylates a number of downstream substrates.[79-82] Yuan *et al.*[83] reported that miR-203 can significantly inhibit the proliferation of OSCC cells through the fjNp63-mediated signal pathway. fjNp63, an alternative splice variant of p63 gene lacking TA domain,[84] is the major isotype expressed in a variety of human squamous cell carcinomas including OSCC,[85] and the expression level of fjNp63 in tumour tissues was significantly higher than in the matched normal tissues.[85,86] Chen *et al.*[56] revealed that miR-92a promotes OSCC cell migration and invasion at least partially via suppression of CDH1 expression, and patients with upregulated miR-92a are prone to lymph node metastasis and thus have a poor prognosis. Kano *et al.*[47] found that miR-145 and miR-133a/b directly regulate FSCN1 and contribute to cellular proliferation and invasion in OSCC. Tsuchiya *et al.*[57] showed that miR-210 mediated mainly by the targeting of fibroblast growth factor receptor-like 1 (FGFRL1), inhibiting the proliferation of OSCC cells by inducing cell cycle arrest and apoptosis. Kong *et al.*[87] reported that downregulation of miR-375, which is mainly caused by promoter methylation, is one of the molecular mechanisms involved in the development and progression of OSCC through inhibiting the expression of IGF1R. Zhang *et al.*[65] found that miR-1322 can regulate oesophageal cancer-related gene 2 (ECRG2) in an allele-specific manner.

MiRNAs AND THEIR TARGET GENES AFTER MEDICAL TREATMENT

Several studies have focused on the alteration and mechanism of miRNAs in the medical treatment of OSCC patients. It was shown that some miRNA alteration was closely related to some medical treatment. For example, Hummel *et al.*[88] revealed that 13 miRNAs (miR-199a-5p, miR-302f, miR-320a, miR-342-3p, miR-425, miR-455-3p, miR-486-3p, miR-519c-5p, miR-548d-5p, miR-617, miR-758, miR-766, miR-1286) were deregulated after 24- and/or 72-hours of treatment (Cisplatin or 5-fluorouracil) in OSCC cell lines. Additionally, many researchers appeared to notice the roles and possible mechanisms of miRNAs in the medical treatment of OSCC. For example, Hong *et al.*[55] described that downregulation of miR-296 could confer sensitivity of both P-glycoprotein-related and P-glycoprotein-nonrelated drugs on OSCC cells, and might promote ADR-induced apoptosis, accompanied by increased accumulation and decreased release of ADR. Imanaka *et al.*[45] showed that miR-141 negatively regulates the expression of YAP1 and conferred cisplatin resistance in OSCC. Hummel *et al.*[48] revealed that miR-148a upregulation sensitises chemotherapy-resistant variants of OSCC cell lines, to cisplatin and 5-FU *in vitro*, and further improves sensitivity in the corresponding chemotherapy-sensitive maternal cell lines. Mitogen and stress-activated protein kinase 1 (MSK1), *de novo* DNA methylation and pregnane x receptor (PXR) were potential mediators of these observations. Hamano *et al.*[89] demonstrated that overexpression of miR-200c induced chemoresistance in OSCC cell lines mediated through activation of the AKT signalling pathway. Hummel *et al.*[61] also suggested that miR-21, miR-106a, and miR-148a correlate with tumour location, distant lymph node metastases and outcome in patients with locally advanced OSCC, and might inform the initial assessment of these patients and predict those at higher risk of postsurgical recurrence. Consequently, it can provide very valuable information to guide us to

evaluate the patient's condition and choose the most fitting medical treatment methods in individual OSCC patients. Furthermore, it could mean that a specific miRNA could have a treatment effect in itself.

CLINICAL APPLICATION OF MiRNAs

Improvement in the survival rate of patients with OSCC will most likely result from new therapeutics based on an increased understanding of the tumour biology and identification of biomarkers for earlier detection. The differential miRNA expression of OSCC may lead to identification of specific markers for progression, molecular classification of carcinoma lesions. Both oncomiRs and tumour suppressor miRs have the potential to serve as molecular therapeutic targets. The inhibition of oncomiRs and the promotion of tumour suppressor miRs should result in increased levels of tumour suppressor proteins or decreased oncogene-induced proteins. For example, the inhibition of miR-21 may possibly regulate PTEN, TPM1, PDCD4, Sprouty2, BCL2, programmed cell and maspin and their corresponding proteins and may inhibit tumour growth, invasion, and metastasis as a result. Upregulating the expression of miR-29c may inhibit the tumour growth through inhibition of cyclin-E. Additionally, we could choose the best medical treatment for individuals by detecting and evaluating specific miRNA. Furthermore, we can change the expression of specific miRNA to improve the treatment effect for some medical methods. For example, the OSCC patients of overexpression of miR-200c may lead the chemoresistance, and inhibition of miR-200c may possibly promote the chemotherapy-sensitivity and improve the effect of chemotherapy if there is no other method to choose.

Consequently, a better understanding of changes in miRNA expression and their functions during OSCC carcinogenesis might lead to possible improvements in the diagnosis and treatment of OSCC.

At present, there are no reports on the use of miRNA for anticancer therapy in the clinical field. However, miRNA therapy provides an attractive antitumour approach for integrated cancer therapy.

CONCLUSION

To the best of our knowledge, miRNA expression and its role in OSCC have considerably advanced our understanding of the molecular mechanisms of OSCC. Emerging reports on miRNAs in OSCC have suggested that miRNAs are promising in stratifying the risk of susceptibility to developing and diagnosing OSCC, and developing future therapeutic targets in OSCC. Furthermore, we can make full use of this knowledge to guide us to evaluate and improve the patient's condition and choose the most fitting medical treatment or explore new approaches to improve the survival rate of OSCC patients.

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