MicroRNA as a molecular target for gastrointestinal cancers

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> **Abstract:** MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally suppress the expression of many target genes, thereby contributing to the modulation of diverse cellular fates in tumor biology. Recent accumulating evidences have demonstrated that aberrant expression of miRNAs is highly associated with tumor initiation, progression and metastasis in various types of cancers including gastrointestinal (GI) cancers. Exploration of precise miRNA-based gene regulatory networks in the pathogenesis of GI cancers will provide important information for the development of novel anticancer strategies aimed at normalizing the critical miRNAs that are deregulated in GI cancers. Recent reports using clinical samples have shown the potential of upregulation of oncogenic miR-21 and of downregulation of the tumor-suppressive miR-34 family as novel molecular biomarkers for the diagnostic and prognostic prediction of GI cancers. In this review, we have focused on the functional roles of these two cancer-related miRNAs, miR-21 and miR-34, that are commonly deregulated during the development and progression of GI cancers. Moreover, the therapeutic potential of miRNA-targeting anticancer therapy is discussed for clinical application as novel anticancer therapy for GI cancers.

Keywords: Gastrointestinal (GI) cancer; microRNA (miRNA); p53; methylation

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Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that consist of approximately 22 nucleotides, and they posttranscriptionally suppress the expression of many target genes by pairing with complementary nucleotide sequences in the 3'-untranslated regions of the target mRNA (1). A number of reports have demonstrated that many kinds of miRNAs regulate diverse cell fates in tumor biology, including cell proliferation (2), cell cycle arrest (3), apoptosis (4), senescence (5), the epithelial-mesenchymal transition (6), invasion and metastasis (7) in human cancer cells, suggesting the potential role of miRNAs in tumor initiation, progression and metastasis. Indeed, aberrant regulation of miRNAs has been frequently reported in a variety of cancers including gastrointestinal (GI) cancers (8,9). Interestingly, a previous report has suggested that GI tumors can be strictly distinguished from non-GI tumors by analysis of global miRNA expression profiles (8). There are two types of miRNAs, oncogenic and tumor-suppressive miRNAs, which are involved in the pathogenesis of GI cancers (10-14). In this review, we have focused on the functional role of two types of cancer-related miRNAs, oncogenic miR-21 (15) (*Figure 1A,B*) and the tumorsuppressive miR-34 family (16) (*Figure 2A,B*). These miRNAs are commonly and frequently deregulated in GI cancers (9,17) and their aberrant expression is associated with the development and progression of GI cancers. Moreover, the potential clinical application of cancerrelated miRNAs as novel molecular biomarkers for cancer therapy of GI cancers is discussed.

miRNAs commonly deregulated in GI cancers

Recent advances in tumor biology have revealed the



Figure 1 Oncogenic function of, and targeting therapy for miR-21 in human gastrointestinal cancers. (A) Chronic inflammation or an inflammatory cytokine activates STAT3 expression, leading to activation of oncogenic miR-21. miR-21 is also upregulated by gain of chromosome 17q23. miR-21 overexpression downregulates target genes (PTEN, CDC25A, FASL, PDCD4 and TGFβR2), contributing to the induction of proliferation, chemoresistance, invasion ability and stemness property and to the suppression of cell cycle arrest and apoptosis. Moreover, miR-21-mediated PTEN suppression activates STAT3 expression as a positive feedback loop; (B) an anti-inflammatory drug or a JAK/STAT3 inhibitor suppresses STAT3 expression, leading to suppression of oncogenic miR-21. miR-21 is also downregulated by miR-21 antisense oligonucleotides and a miR-21 sponge. miR-21 suppression upregulates target genes (PTEN, CDC25A, FASL, PDCD4 and TGFβR2), contributing to suppression of proliferation, chemoresistance, invasion ability and stemness properties and to induction of cell cycle arrest and apoptosis. Moreover, upregulation of the miR-21-target PTEN suppresses STAT3 expression as a negative feedback loop. miRNA, microRNA; STAT3, signal transducer and activator of transcription 3; PTEN, phosphatase and tensin homologue; CDC25A, cell division cycle 25A; FASL, FAS ligand; PDCD4, programmed cell death 4; TGFβR2, transforming growth factor beta receptor 2.

aberrant expression of many miRNAs in a variety of human cancers including GI cancers, suggesting a potential role of miRNAs in tumor initiation, progression and metastasis. Indeed a number of reports have indicated that miRNAs can regulate diverse cell fates in human cancer cells. In various cancer tissues, deregulation of miRNAs has been shown to be highly associated with transcriptional deregulation, mutations, epigenetic methylations, DNA copy number abnormalities and defects in the miRNA biogenesis machinery (18). Among the many kinds of miRNAs, two cancer-related miRNAs, miR-21 and miR-34, have been shown to be commonly and frequently deregulated in GI cancer tissues (9,12,17). Oncogenic miR-21 is upregulated and tumor-suppressive miR-34 is downregulated by genetic and epigenetic alterations and by an inflammatory microenvironment in human GI cancers.

Expression, regulation and oncogenic function of miR-21 in GI cancers

miR-21 is frequently upregulated in tumor tissues of a variety of human GI cancers compared to normal tissues (*Table 1*). Human esophageal cancers including squamous cell carcinomas and adenocarcinomas showed significantly increased miR-21 expression (19-23) in association with clinical stage (20,21) and lymph node metastasis (21). In human gastric cancers, miR-21 was upregulated (24-28) and its overexpression was significantly associated with tumor



Figure 2 Tumor-suppressive function of, and targeting therapy for miR-34 in human gastrointestinal cancers. (A) Genetic mutation or loss of chromosome 17p13 suppresses p53 expression, leading to suppression of the tumor-suppressive miR-34. miR-34 is also downregulated by methylation and SNPs of the promoter region and by loss of chromosomes 1p36 and 11q23. miR-34 suppression upregulates target genes (CDK6, MYC, BCL2, E2F3, SNAIL and NOTCH1), contributing to induction of proliferation, invasion ability and stemness properties and to suppression of cell cycle arrest, apoptosis and senescence. Moreover, upregulation of the miR-34-target SIRT1 suppresses p53 expression as a negative feedback loop; (B) chemotherapy or radiotherapy activates p53 expression, leading to activation of the tumor-suppressive miR-34. p53 is also upregulated by Ad-p53 and CRAd-p53. miR-34 is directly upregulated by MRX34 and by a demethylator. miR-34 overexpression downregulates target genes (CDK6, MYC, BCL2, E2F3, SNAIL and NOTCH1), contributing to the suppression of proliferation, invasion ability and stemness property and to the induction of cell cycle arrest, apoptosis and senescence. Moreover, miR-34 overexpression downregulates target genes (CDK6, MYC, BCL2, E2F3, SNAIL and NOTCH1), contributing to the suppression of proliferation, invasion ability and stemness property and to the induction of cell cycle arrest, apoptosis and senescence. Moreover, miR-34-mediated SIRT1 suppression activates p53 expression as a positive feedback loop. miRNA, microRNA; CDK6, cyclin-dependent kinase 6; BCL2, B-cell CLL/lymphoma 2; E2F3, E2F transcription factor 3; SNAIL, snail family zinc finger 1; Ad-p53, p53-expressing adenovirus; CRAd-p53, conditionally replicating p53-expressing adenovirus; SNP, single nucleotide polymorphism.

size (27), clinical stage (27), and poor relapse-free and overall survival (28). Human colon cancers showed miR-21 upregulation (29-34), which was associated with clinical stage (29,31), metastatic activity (29,30) and poor survival (31-34). Colorectal adenoma also exhibited significant miR-21 upregulation (31). In human pancreatic cancers, miR-21 expression was significantly higher than normal controls (35-41) and miR-21 upregulation was significantly correlated with metastatic behavior (38,40), recurrence (41) and poor survival (38,39,41). Human liver cancers showed significantly higher miR-21 expression (42-46), which was significantly associated with liver cirrhosis (45), clinical stage (45,46) and poor prognosis (45,46). Recent evidences also support the potential of miR-21 overexpression as a prognostic marker in other types of cancers (51). More interestingly, some recent reports have further focused on miR-21 upregulation in tumor stromal fibroblasts in human GI cancers (47-50). Stromal miR-21 overexpression was associated with tumor size (47), clinical stage (47), lymph node metastasis (47,49), shorter disease-free survival (48), and overall survival (50). These findings suggest a functional role for miR-21 overexpression in both tumor cells and stromal fibroblasts in the development and progression of GI cancers.

Two possible molecular mechanisms have been proposed for the upregulation of miR-21 in human GI

Table 1 Upregula	tion of mik	R-21 expression	on in tumor and	stromal tiss	sues from	patients wi	th GI cance	ers			
	locial	Alimbor of				Clini	copatholog	ical factors			
Cancer type	sample	patients	level -	lumor size	Stage	LN meta	Distant meta	Recurrence	Poor survival	Other diseases	References
Esophageal	Tumor	16	Upregulation	I	I	I	I	I	I	I	Wijnhoven <i>et al.</i> (19)
cancer	Tumor	38	Upregulation	I	S	I	I	I	I	I	Mori <i>et al.</i> (20)
	Tumor	76	Upregulation	I	ა	S	I	I	I	I	Li <i>et al.</i> (21)
	Tumor	170	Upregulation	I	I	I	I	I	I	I	Mathé <i>et al.</i> (22)
	Tumor	178	Upregulation	I	I	I	I	I	I	I	Zhao <i>et al.</i> (23)
Gastric cancer	Tumor	c	Upregulation	I	I	I	I	I	I	I	Guo <i>et al.</i> (24)
	Tumor	10	Upregulation	I	I	I	I	I	I	I	Zhang <i>et al.</i> (25)
	Tumor	37	Upregulation	I	I	I	I	I	I	I	Chan <i>et al.</i> (26)
	Tumor	49	Upregulation	ა	ა	I	I	I	I	I	Motoyama <i>et al.</i> (27)
	Tumor	100	Upregulation	I	I	I	I	I	ა	I	Li <i>et al</i> . (28)
Colorectal cancel	r Tumor	29	Upregulation	I	ა	S	S	I	I	I	Slaby et al. (29)
	Tumor	82	Upregulation	I	I	თ	S	I	I	I	Drusco <i>et al.</i> (30)
	Tumor	84	Upregulation	I	ა	I	I	I	ა	Adenoma	Schetter et al. (31)
	Tumor	115	Upregulation	I	I	I	I	I	ა	I	Schetter et al. (32)
	Tumor	301	Upregulation	I	I	I	I	I	S	I	Oue <i>et al.</i> (33)
	Tumor	764	Upregulation	I	I	I	I	I	S	I	Kjaer-Frifeldt et al. (34)
Pancreatic	Tumor	18	Upregulation	I	I	I	I	I	I	I	Tavano <i>et al.</i> (35)
cancer	Tumor	25	Upregulation	I	I	I	I	I	I	I	Moriyama <i>et al.</i> (36)
	Tumor	28	Upregulation	I	I	I	I	I	I	I	Lee <i>et al.</i> (37)
	Tumor	65	Upregulation	I	I	თ	I	I	S	I	Nagao <i>et al.</i> (38)
	Tumor	80	Upregulation	I	I	I	I	I	ა	I	Dillhoff <i>et al.</i> (39)
	Tumor	81	Upregulation	I	I	I	S	I	I	I	Giovannetti <i>et al.</i> (40)
	Tumor	127	Upregulation	I	I	I	I	ა	S	I	Hwang <i>et al.</i> (41)
Liver cancer	Tumor	က	Upregulation	I	I	I	I	I	I	I	Meng <i>et al.</i> (42)
	Tumor	43	Upregulation	I	I	I	I	I	I	I	Jiang <i>et al.</i> (43)
	Tumor	55	Upregulation	I	I	I	I	I	I	I	Ladeiro <i>et al.</i> (44)
	Tumor	60	Upregulation	I	ა	I	I	I	S	iver cirrhosis	Karakatsanis et al. (45)
	Tumor	119	Upregulation	I	ა	I	I	I	S	I	Wang <i>et al.</i> (46)
Gastric cancer	Stroma	454	Upregulation	S	S	S	I	I	I	I	Uozaki <i>et al.</i> (47)
Colorectal cancel	r Stroma	197	Upregulation	I	I	I	I	I	S	I	Nielsen <i>et al.</i> (48)
Pancreatic	Stroma	153	Upregulation	I	I	ა	I	I	I	I	Kadera <i>et al.</i> (49)
cancer	Stroma	181	Upregulation	I	I	I	I	I	ა	I	Donahue <i>et al.</i> (50)
miRNA, microRN	A; GI, gast	rointestinal;	S, significant re	elationship.							

Tazawa et al. Role of miRNA in GI cancers

cancer cells: (I) an inflammatory environment; and (II) chromosomal amplification (Figure 1A). Examples of (I) are the significantly higher miR-21 expression in Helicobacter pylori-infected gastric mucosa (25) and the up-regulation of miR-21 expression by the hepatitis B virus X protein (52). Moreover, several inflammatory cytokines have recently been shown to be responsible for miR-21 upregulation (32). miR-21 is upregulated by interleukin-6 (IL-6)-dependent induction of signal transducer and activator of transcription 3 (STAT3) in human colon cancer cells (53). These results strongly suggest a relationship between miR-21 upregulation and an inflammatory microenvironment. With regards to (II), chromosomal instability has also been suggested to be associated with high miR-21 expression. For example, miR-21 is located on human chromosome 17q23, which is frequently amplified in GI cancers (54). These combined findings demonstrate that miR-21 is frequently overexpressed through inflammatory stimuli and chromosomal instability in GI cancers.

Regarding the molecular mechanism of the oncogenic function of miR-21 (Figure 1A), miR-21 overexpression promotes cell proliferation in human GI cancers through suppression of the phosphatase and tensin homologue (PTEN) gene (42,55,56). miR-21-mediated suppression of PTEN upregulates STAT3 expression through activation of the IL-6 signaling pathway (53) as a positive feedback loop. miR-21 induces cell cycle progression by suppressing cell division cycle 25A (CDC25A) in human colon cancer cells (57). miR-21 further functions as an anti-apoptotic factor by suppressing the pro-apoptotic Fas ligand (FASL) (58). miR-21-mediated chemoresistance (59) and invasion ability (60,61) are induced by suppressing the tumor-suppressive programmed cell death 4 (PDCD4) gene, whose expression is significantly downregulated in human GI cancers (62). miR-21 overexpression also induces stemness properties by suppressing transforming growth factor beta receptor 2 (TGF β R2) in human colon cancer cells (63). Thus, miR-21 overexpression is highly associated with the development and progression of human GI cancers through suppression of multiple tumorsuppressive signaling pathways.

Expression, regulation and tumor-suppressive function of the miR-34 family in GI cancers

The expression levels of the miR-34 family in various types of cancers including GI cancers have been summarized in a recent review (64). This review indicates that the miR-34 family can be upregulated or downregulated in human cancer tissues. We previously reported that miR-34a expression was downregulated in 9 (36%) out of 25 human colon cancer tissues compared with the corresponding normal tissues (5). Since the miR-34 family (miR-34a, -34b and -34c) is a family of tumor suppressive miRNAs that are mainly induced by the tumor suppressor p53 gene (5,17,65-68), the miR-34 family may be upregulated by DNA damage in p53-activated tumor cells and downregulated by genetic and epigenetic alterations in p53-inactivated tumor cells (12). Three possible molecular mechanisms have been proposed for miR-34 miRNA regulation, especially for the downregulation of miR-34 family members, in human GI cancer tissues (Figure 2A): (I) p53 dysfunction; (II) methylation and single nucleotide polymorphisms (SNPs) in the promoter region; (III) chromosomal deletion. Regarding (I), dysfunction of the tumor suppressor p53 is frequently observed by mutation (69-71) or by deletions of chromosome 17p13 (72-74), on which the p53 gene is located, in more than 50% of human GI cancers. Regarding (II), a variety of human cancer cells including gastric cancers exhibit frequent hypermethylation of the miR-34a promoter (75). The expression of miR-34b/c is also downregulated through promoter hypermethylation in human colon cancer tissues and cell lines, although normal colon tissues show no methylation (76). Moreover, some recent reports have suggested a possible relationship between the SNP rs4938723 of the miR-34b/c promoter region and the risk of colorectal cancers (77) and liver cancers (78-80). Regarding (III), the location of miRNA on human chromosomes has been reported to be associated with the fragile chromosomal sites that have been detected in a variety of human cancers (81). In fact, miR-34a is located on human chromosome 1p36, which is frequently deleted in GI cancers (82). miR-34b/c is located on human chromosome 11q23, which is a fragile site that is associated with breast and lung cancers (81) and which has been identified as a colorectal cancer susceptibility locus in a genome-wide association study (83). These accumulating evidences strongly suggest that the expression of the miR-34 family is frequently downregulated through p53 dysfunction, genetic and epigenetic alterations of the promoter region and chromosomal instability in GI cancers.

As for the molecular mechanism of the tumor-suppressive function of the miR-34 family (*Figure 2B*), miR-34 suppresses cyclin-dependent kinase 6 (CDK6) expression resulting in inhibition of proliferation (84), MYC expression, resulting in cell cycle arrest (85), BCL2 (B-cell CLL/lymphoma 2) expression, resulting in apoptosis induction (86), E2F3 (E2F transcription factor 3) expression, resulting in senescence induction (5), SNAIL (snail family zinc finger 1) expression, resulting in inhibition of invasion (87), and NOTCH1 expression, resulting in inhibition of stemness properties (88) in human cancer cells. We previously showed that miR-34a overexpression causes not only downregulation of E2F-related genes, but also upregulation of p53-related genes in human colon cancer cells (5), suggesting that miR-34 overexpression can activate the p53-signaling pathway probably through a positive feedback loop. In fact, it has been shown that suppression of SIRT1 (sirtuin 1) expression by miR-34a induces p53 activation and subsequent upregulation of p53-downstream target genes including p21 as a positive feedback loop (89) (Figure 2B). Thus, the p53-miR-34 regulatory network suppresses multiple oncogenic signaling pathways and the development and progression of GI cancers. In contrast, downregulation of the miR-34 family contributes to the activation of many miR-34-target oncogenes through SIRT1-mediated negative feedback loop (Figure 2A).

miRNAs as diagnostic and predictive biomarkers for GI cancers

Blood is a very useful sample as a non-invasive liquid biopsy for cancer patients. Circulating nucleic acids, including DNA, mRNA and miRNA in blood samples have emerged as having great potential as novel molecular biomarkers for cancer patients (90). In particular, it has recently been shown that detection of oncogenic miRNA overexpression in blood samples such as plasma and serum, is likely to be a useful method for the early diagnosis and prognostic prediction of various types of cancers (91). In contrast, tumor-suppressive miRNAs are often downregulated due to promoter hypermethylation during the pathogenesis of cancer development. The detection of promoter methylation status and the downregulation of tumor-suppressive miRNAs in tumor tissues is a promising biomarker for the diagnosis and prognosis of GI cancers. Moreover, stool samples also have potential as a non-invasive sample for detection of the DNA methylation status of GI cancer patients (92).

Upregulation of oncogenic miR-21 in blood and stool samples

Recent accumulating evidences have suggested the diagnostic and prognostic potential of circulating oncogenic

miR-21 in blood samples from cancer patients (93). The expression levels of oncogenic miR-21 in plasma and serum were significantly higher in patients with esophageal cancers (94-97), gastric cancers (96,98-102), colorectal cancers (96,103-107), pancreatic cancers (108-114), and liver cancers (115-117) compared to healthy controls (Table 2). High miR-21 expression in plasma and serum is significantly associated with tumor size (95,98,102,105), clinical stage (97,98,100-102,104,105,108), metastatic activity (98,99,101,105,106,108), recurrence (94) and poor survival (105,108,110,113). Recently, the isolation of exosomes has emerged as a useful method for the detection of high miR-21 expression in serum from GI cancer patients (97,107,114,117). Some reports have shown the downregulation of high miR-21 expression after operation or chemotherapy, strongly supporting the possibility of tumor-derived circulating miR-21. miR-21 expression was also high in blood samples from patients with precancerous diseases, such as colorectal adenoma (105), chronic hepatitis B (116) and liver cirrhosis (117). In contrast, in stool samples, the expression of miR-21 was higher in patients with colorectal cancers compared to healthy controls (118-121), although pancreatic cancer patients did not show significant increases in miR-21 expression (122,123). These evidences suggest that detection of oncogenic miR-21 overexpression in blood samples is a promising screening system for the early diagnosis and prognostic prediction of GI cancers. Moreover, the isolation of miRNA using stool samples would be a useful method for the detection of miR-21 overexpression, especially in colorectal cancer patients.

Detection of the downregulation of the tumorsuppressive miR-34 family by promoter hypermethylation in tumor and stool samples

Hypermethylation of the promoters of tumor-suppressor miR-34 family members has been frequently observed in GI tumor tissues (*Table 3*). The incidence of miR-34a promoter methylation is moderately high (13.0-79.3%) in GI cancers (75,124-129), although the incidence of miR-34a promoter methylation in gastric cancers remains unclear. In contrast, the incidence of miR-34b/c promoter methylation was quite high (40.7-100.0%) in GI cancers (76,124,126-133). Moreover, the incidence of promoter methylation in the miR-34 family was also high (75.0-93.6%) in stool samples from colorectal cancer patients (128,133) and was similar to the methylation in tumor tissues. The promoter hypermethylation of miR-34 family

Table 2 U _I	pregulation	of miR-	-21 in blood sam	ples fro	om patie	nts with	n GI cancer	S					
10000		Numbe	er Everacion			Clin	icopatholo	gical factor	S		Trontmont		
type	sample	of patient:	L Level 1	lumor size	Stage	LN meta	Distant _R meta	ecurrence	Poor survival	Other diseases	Response	Treatment	References
Esophage	al Plasma	50	Upregulation	I	I	I	I	S	I	I	Downregulation	Operation	Komatsu et al. (94)
cancer	Serum	71	Upregulation	S	I	I	I	I	I	I	Downregulation	Operation,	Kurashige <i>et al.</i> (95)
											0	chemotherapy	
	Serum		Upregulation	I	I	I	I	I	I	I	I	I	Wang <i>et al</i> . (96)
	Exosome (Serum)	51	Upregulation	I	ა	I	1	I	I	I	I	I	Tanaka <i>et al.</i> (97)
Gastric	Blood	53	Upregulation	თ	ა	S	S	I	I	I	I	I	Zheng <i>et al.</i> (98)
cancer	Plasma	42	Upregulation	I	I	S	I	I	I	I	Downregulation	Operation	Ma <i>et al.</i> (99)
	Plasma	70	Upregulation	I	ა	I	I	I	I	I	I	I	Li <i>et al.</i> (100)
	Serum	30	Upregulation	I	I	I	I	I	I	I	I	I	Wang <i>et al.</i> (96)
	Serum	79	Upregulation	I	S	S	I	I	I	I	I	I	Kim <i>et al.</i> (101)
	Serum	103	Upregulation	ა	S	I	I	I	I	I	I	I	Song <i>et al.</i> (102)
Colorectal	Plasma	50	Upregulation	I	I	I	I	I	I	I	I	I	Kanaan <i>et al.</i> (103)
cancer	Serum	32	Upregulation	I	I	I	I	I	I	I	I	I	Wang <i>et al.</i> (96)
	Serum	40	Upregulation	I	ა	I	I	I	I	I	I	I	Basati <i>et al.</i> (104)
	Serum	186	Upregulation	ა	ა	I	S	I	S	Adenoma	I	I	Toiyama <i>et al.</i> (105)
	Serum	224	Upregulation	I	I	I	S	I	I	I	I	I	Yin <i>et al.</i> (106)
	Exosome (Serum)	88	Upregulation	I	I	I	I	I	I	I	Downregulation	Operation	Ogata-Kawata <i>et al.</i> (107)
Pancreatic	Plasma	32	Upregulation	I	S	S	S	I	S	I	I	I	Abue <i>et al.</i> (108)
cancer	Plasma	49	Upregulation	I	I	I	I	I	I	I	I	I	Wang <i>et al.</i> (109)
	Plasma	50	Upregulation	I	I	I	I	I	S	I	I	I	Ali <i>et al.</i> (110)
	Plasma	138	Upregulation	I	I	I	I	I	I	I	I	I	Liu <i>et al.</i> (111)
	Serum	35	Upregulation	I	I	I	I	I	I	I	I	I	Kong <i>et al.</i> (112)
	Serum	197	Upregulation	I	I	I	I	I	S	I	I	I	Liu <i>et al.</i> (113)
	Exosome (Serum)	22	Upregulation	I	I	I	I	I	I	I	I	I	Que <i>et al.</i> (114)
Liver	Serum	46	Upregulation	I	I	I	I	I	I	I	I	I	Li et al. (115)
cancer	Serum	101	Upregulation	I	I	I	I	I	I	Chronic	I	I	Xu <i>et al.</i> (116)
									_	nepatitis B			
	Exosome (Serum)	30	Upregulation	I.	ი	I.	I	I	I	Liver cirrhosis	I	I	Wang <i>et al.</i> (117)
GI, gastroii	ntestinal; m	iRNA,	microRNA; S, si	gnifica	ant relat	ionship			L				

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Table 3 De	stection of promoter	r methylatio	n of miR-34 fami	ly members in tume	or and stool s	amples from p	atients with GI cand	ters	
miRNA	Cancer type	Clinical sample	Number of total patients	Number of positive patients	Incidence (%)	Promoter methvlation	Expression level	Significant clinical factors	References
miR-34a	Esophageal	Tumor	54	. 36	66.7	Yes	Downregulation	I	Chen <i>et al.</i> (124)
	cancer	Tumor	59	6	15.3	Yes	Downregulation	Stage, LN meta	Cui <i>et al.</i> (125)
	Colorectal	Tumor	23	ო	13.0	Yes	Downregulation	I	Lodygin <i>et al.</i> (75)
	cancer	Tumor	114	84	73.7	Yes	Downregulation	I	Vogt <i>et al</i> . (126)
		Tumor	93	42	45.1	Yes	Downregulation	LN meta, Distant meta	Siemens et al. (127)
		Tumor	82	65	79.3	Yes	Downregulation	LN meta	Wu <i>et al.</i> (128)
	Pancreatic	Tumor	19	က	15.7	Yes	Downregulation	I	Lodygin <i>et al.</i> (75)
	cancer	Tumor	11	7	63.6	Yes	Downregulation	I	Vogt <i>et al</i> . (126)
	Liver cancer	Tumor	43	31	72.1	Yes	Downregulation	I	Xie <i>et al.</i> (129)
miR-34b/c	Esophageal cancer	Tumor	54	22	40.7	Yes	Downregulation	I	Chen <i>et al.</i> (124)
	Gastric cancer	Tumor	118	83	70.3	Yes	Downregulation	I	Suzuki <i>et al.</i> (130)
		Tumor	72	42	58.3	Yes	Downregulation	Stage	Tsai <i>et al.</i> (131)
		Tumor	28	19	67.9	Yes	Downregulation	I	Du <i>et al.</i> (132)
	Colorectal	Tumor	111	101	91.0	Yes	Downregulation	I	Toyota <i>et al</i> . (76)
	cancer	Tumor	114	113	99.1	Yes	Downregulation	I	Vogt <i>et al</i> . (126)
		Tumor	82	62	97.5	Yes	Downregulation	I	Kalimutho <i>et al.</i> (133)
		Tumor	86	62	91.9	Yes	Downregulation	I	Siemens et al. (127)
		Tumor	82	80	97.5	Yes	Downregulation	I	Wu <i>et al.</i> (128)
	Pancreatic	Tumor	1	11	100.0	Yes	Downregulation	I	Vogt <i>et al</i> . (126)
	cancer								
	Liver cancer	Tumor	43	34	79.1	Yes	Downregulation	I	Xie <i>et al.</i> (129)
miR-34a	Colorectal	Stool	82	63	76.8	Yes	Downregulation	I	Wu <i>et al.</i> (128)
	cancer								
miR-34b/c	Colorectal	Stool	28	21	75.0	Yes	Downregulation	I	Kalimutho <i>et al.</i> (133)
	cancer	Stool	79	74	93.6	Yes	Downregulation	I	Wu <i>et al.</i> (128)
GI, gastroii	ntestinal; miRNA, n	nicroRNA.							

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Tazawa et al. Role of miRNA in GI cancers

members was associated with their downregulation, which significantly correlated with clinical stage (125,131), lymph node metastasis (125,127,128) and distant metastasis (127). Recent accumulating evidences strongly suggest that promoter hypermethylation of the miR-34 family is a frequent epigenetic alteration during the development and progression of GI cancers, and that tumor and stool samples are useful for detection of the expression and methylation status of the miR-34 family.

miRNAs as novel molecular targets for GI cancers

Since the expression of miR-21 and the miR-34 family is commonly and frequently dysregulated in human GI cancer tissues (Tables 1,3), these findings suggest that miR-21 and the miR-34 family are promising molecular targets for the treatment of patients with GI cancers. Human GI cancers show miR-21 upregulation, which is induced by inflammatory stimuli, STAT3 activation and chromosomal instability. miR-21 overexpression functions as oncogenic miRNA during tumor development and progression. In contrast, the miR-34 family members are downregulated by p53 dysfunction, promoter hypermethylation and chromosomal instability in human GI cancers. Downregulation of the miR-34 family contributes to cell proliferation, cell cycle progression, invasion and metastasis. Based on the molecular mechanism of the regulation of oncogenic miR-21 and the tumor-suppressive miR-34 family, several kinds of therapeutic options for miR-21suppressing therapy and miR-34-activating therapy could be developed.

Therapeutic potential of miR-21-suppressing therapy

Since a variety of human cancer cells including GI cancers have been shown to overexpress miR-21 (24-26,28), the development of miR-21-target therapy that suppresses oncogenic miR-21 overexpression is a promising antitumor therapy against miR-21-overexpressing human cancers. There are several strategies for the suppression of oncogenic miR-21 upregulation in human cancer cells, such as antiinflammatory drugs, a STAT3 inhibitor, miR-21 antisense oligonucleotides and miR-21 sponges (*Figure 1B*). To suppress inflammation-mediated miR-21 upregulation, antiinflammatory drugs may be a useful option. For example, the anti-inflammatory drug, curcumin, has been shown to downregulate miR-21 expression in human pancreatic cancer cells (134). Curcumin inhibits IL-6-mediated STAT3 activation (135), which probably leads to miR-21 upregulation in human colon cancer cells (53). Curcumin treatment may downregulate inflammation-induced miR-21 upregulation in GI cancers. To suppress STAT3-mediated miR-21 upregulation more strongly than curcumin, inhibitors of STAT3 and JAK, which is a STAT3-activating kinase, may be useful reagents (136-140). In contrast, to suppress miR-21 upregulation due to chromosome 17q23 gain, miR-21 antisense oligonucleotides or a miR-21 sponge may be effective. miRNA antisense oligonucleotides have been frequently used to directly and specifically suppress the expression of oncogenic miRNAs in preclinical experiments. In fact, a miR-21 antisense oligonucleotide has been shown to suppress miR-21 expression in human gastric cancer cells, resulting in suppression of cell proliferation and induction of apoptotic cell death (25). Recently, a biopharmaceutical company Regulus Therapeutics Inc. is planning a clinical trial of RG-012, which is an anti-miR targeting miR-21, for the treatment of renal dysfunction in Alport syndrome patients (Table 4). Moreover, a miRNA sponge, which contains multiple binding sites for a specific miRNA, is also expected to downregulate the inhibitory effect of endogenous miRNAs against many target genes (141). It has recently been shown that a miRNA sponge for oncogenic miR-10b, whose expression is significantly associated with metastasis of breast cancers, can suppress miR-10b as efficiently as an antisense oligonucleotide (142). However, the therapeutic potential of a miRNA sponge for oncogenic miR-21 in GI cancers remains unclear. These reports suggest that the use of anti-inflammatory drugs, STAT3/JAK inhibitors, miR-21 antisense oligonucleotides or a miR-21 sponge are promising anticancer strategies for the suppression of oncogenic miR-21 overexpression in GI cancers.

Therapeutic potential of miR-34-activating therapy

For activation of the miR-34 family in human GI cancers, the status of p53 and miR-34 abnormalities should be considered (12). In both p53- and miR-34-intact human cancer cells, conventional anticancer therapy, such as chemotherapy and radiotherapy, efficiently induces miR-34 expression through activation of endogenous p53 (*Figure 2B*) (5,65-68). However, since more than 50% of human GI cancers lack normal p53 function and are therefore deficient in p53-induced miR-34 expression, novel

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Table 4 (Clinical studies	of miR-21-suppressing	g and miR-34-activatin	ng therapie	S			
Drug	miRNA regulation	Disease	Therapy type	Phase of study	Estimated enrollment of patient	Country	Sponsor	NIH identifier
RG-012	miR-21 suppression	Alport syndrome	Monotherapy	Ι	Unknown	USA	Regulus Therapeutics, Inc.	-

Monotherapy

48

I

USA.

Korea

Mirna

Inc.

Therapeutics,

anticancer strategies that can induce miR-34 expression in p53-inactivated tumors should be considered. For induction of miR-34 expression in human cancer cells in which p53 is inactivated due to mutation or to chromosome 17p13 loss, infection with exogenous p53-expressing adenovirus (Ad-p53) vectors would be a useful method (Figure 2B). Preclinical studies have shown that a replication-deficient Ad-p53 vector suppresses cell proliferation and tumor growth through p53-mediated induction of apoptotic cell death in human gastric cancer cells (143,144). We previously reported that Ad-p53-mediated wild-type p53 transfer efficiently suppressed cell proliferation, tumor growth and angiogenesis in human colon cancer cells (145,146). A phase I clinical trial has shown that treatment with Ad-p53 was well tolerated in patients with advanced esophageal cancers (147). However, the low transduction rate of p53 gene transfer by the replication deficient Adp53 is a major problem that needs to be overcome in order to improve the clinical outcome in patients with advanced GI cancers. We recently reported that combination therapy of Ad-p53 with a replication-competent oncolytic adenovirus enhances and sustains the expression level of the p53 protein, leading to enhanced apoptotic cell death of human colon cancer cells (148). Furthermore, a tumorspecific conditionally replicating Ad-p53 (CRAd-p53) has been shown to enhance and sustain p53 gene expression more efficiently than Ad-p53 (149-151), which probably contributed to strong miR-34 induction in the infected human cancer cells. However, in order to induce miR-34 expression in human cancer cells in which miR-34 is inactivated due to miR-34 family promoter methylation and/or loss of chromosomes 1p36 and 11q23, direct miR-34 upregulation by miR-34 mimics rather than p53 replacement therapy should be attempted (Figure 2B). We previously reported that ectopic expression of miR-34a

Primary and metastatic

malignancies

liver tumors, hematologic

suppressed cell viability and induced subsequent senescence-like growth arrest in human colon cancer cells with either wild-type or mutant p53 protein (5). Interestingly, miR-34a overexpression was recently reported to suppress the stemness properties of p53-mutant human gastric cancer cells (86), human colon cancer stem cells (88), and human pancreatic cancer stem cells (152). These findings strongly suggest that miR-34-based anticancer therapy can target cancer stem cells within GI cancer tissues (153). Recently, a phase I clinical trial of MRX34, which is an miR-34 mimic that induces miR-34 expression following introduction into cells by a liposome delivery system, has been conducted by Mirna Therapeutics, Inc. as a monotherapy in patients with advanced liver cancers (154) (Table 4). In the future, exploration of the antitumor effect of miR-34-based anticancer therapy will shed light on the development of novel anticancer strategies against GI cancers. Moreover, human cancer cells in which miR-34 is inactivated by promoter methylation of the miR-34 family would be further sensitive to demethylating agents, although other kinds of miRNAs may be also reactivated after demethylating therapy.

Future direction of research on miR-21 and miR-34 family

Genetic and epigenetic analyses using clinical samples have shown that oncogenic miR-21 is upregulated and tumorsuppressive miR-34 family is downregulated in most GI cancers (*Tables 1-3*). More understanding of the precise molecular mechanism underlying miR-21 upregulation and miR-34 downregulation would be needed to develop the miRNA-based anticancer therapy. Recently, clinical studies of miR-21-suppressing and miR-34-activating therapies have been conducted to confirm the safe and feasibility

MRX34

miR-34

miRNA, microRNA.

activation

of miRNA-targeting strategy (*Table 4*). For the clinical application of miRNA-targeting therapy, we should confirm the therapeutic potential of miRNA normalization therapy in combination with conventional anticancer therapy, such as chemotherapy and radiotherapy. Moreover, the identification of drugs that suppress miR-21 and/or activate miR-34 would provide novel insights in developing the multimodal antitumor strategy targeting miRNA expression.

Conclusions

Previous studies by many cancer researchers have revealed that diverse genetic and epigenetic alterations in proteincoding genes play central roles in the pathogenesis of GI cancers. However, since non-coding miRNAs have been shown to be deregulated in a variety of human cancers including GI cancers (8,9), it is now also necessary to understand the miRNA-based pathogenesis of GI cancers and the molecular mechanism of the interaction between protein-coding genes and non-coding miRNA genes (155). The development of an early detection system for oncogenic miR-21 and promoter methylation of the tumor-suppressive miR-34 family using clinical samples, such as tumor, blood and stool, would improve the clinical outcome of patients with GI cancers. p53 replacement therapy using Ad-p53 and CRAd-p53 is a promising anticancer therapy against GI cancers with p53 dysfunction. Adenovirus-mediated overexpression of tumor suppressor p53 may further suppress oncogenic miR-21 expression through suppression of STAT3 expression (156,157). In contrast, in human GI cancers with miR-34 dysfunction, direct restoration of miR-34 using miR-34 mimics would be a more effective strategy than p53 replacement therapy for efficient induction of miR-34 expression. As a recent report suggested that combination therapy with miR-34 mimics and KRAS siRNA, which may suppress miR-21 expression (158), has great therapeutic potential in human lung cancer cells in both in vitro and in vivo settings (159), the combination of miR-34activating therapy and miR-21-suppressing therapy may be a promising antitumor strategy. Thus, an understanding at the molecular level of miRNA-mediated cancer progression would provide a novel platform for the development of miRNA-based cancer diagnosis and anticancer therapy for the treatment of patients with GI cancers.

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