

Review

miR-196, an Emerging Cancer Biomarker for Digestive Tract Cancers

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Abstract

Over the past decade, the emergence of microRNA (miRNA) research has firmly established this molecular family as a key component in cells. MiRNAs, which function as negative gene regulators, participate in multiple biological processes and maintain homeostasis in cells. The dysregulation of miRNA may contribute to numerous human disorders, including cancer. Recently, miR-196 was found to be aberrantly expressed in a wide range of malignant diseases, which suggests that it plays important roles in carcinogenesis. Here, we summarize the current knowledge concerning miR-196 family in cancers. This review includes miR-196 gene structure and aberrant expression in various cancers, and current understanding of numerous functions and regulatory targets of miR-196 in specific cancers. Since miR-196 are consistently found over-expressed in digestive tract cancer tissues, we also reviewed the clinical significance and potential applications of miR-196 in these cancers. We highlight that miR-196 may serve as an emerging cancer biomarker for digestive tract cancers.

Key words: miR-196, cancer, clinical application, regulatory mechanism.

Introduction

Over the past decade, the emergence of miRNA research has firmly established this molecular family as a key component in cells. Under normal physiological conditions, miRNAs implement feedback mechanisms by gene transcripts to regulate cell proliferation, differentiation and apoptosis [1-3]. The dysregulation of miRNAs may facilitate malignant transformation, including tumor initiation, growth, invasion, and therapeutic resistance [1-3]. Recently, several investigations have reported that miR-196 is frequently altered in various cancers, which suggests that it plays an important role in carcinogenesis. Here, we review recent reports on miR-196 family molecules in cancer, whose topics include gene structure, biological functions, and

regulatory targets and clinical significance. We highlight that miR-196 may serve as an emerging cancer biomarker for digestive tract cancers.

miRNA discovery, biogenesis and mechanism

miRNAs are endogenous, non-coding small RNA that are approximately 22 nt in length. After the first miRNA was discovered in *C. elegans*, in 1993, the knowledge of miRNA grew rapidly [1]. Currently, more than 2,500 potential human miRNAs are recorded in the miRBase, and more than 30% of all genes are estimated to be regulated by miRNAs. This family of molecules has important and broad

functions in cells, including proliferation, differentiation and apoptosis [2-6].

MiRNAs are encoded in the genome. Their long primary transcripts are produced by RNA polymerase II and are further processed by Drosha and DGCR8, which results in 80- to 100-nt precursor miRNAs (pre-miRNAs) [7, 8]. The pre-miRNAs are transported to the cytoplasm by Exportin5 and are cleaved by Dicer to form double-stranded miRNA duplexes. After unwinding into single-stranded mature miRNAs, the miRNAs bind to Argonaute (Ago2) protein and form the RNA-induced silencing complex (RISC). This complex then binds to the target-gene mRNA, and carries out mRNA degradation or translational repression [2, 9]. More specifically, the mature miRNA recognizes its complementary sequence in the target mRNA 3'-untranslational region (3'-UTR) via the "seed" region of the 5'-miRNA at nucleotide positions 2-7. Recent reports have revealed that the target mRNAs may also be repressed by miRNAs through binding to the coding regions or 5'-UTR [10, 11].

MiRNAs play as negative gene regulators in cells and function based on their target transcripts. Through algorithm analysis of miRNA target prediction, plenty of miRNA-gene relationships have been presumed [12, 13]. Increasing evidences have shown that a single miRNA is able to regulate several transcripts, and a transcript may have multiple miRNA binding sites which allow several miRNAs to bind at a same time [14]. This phenomenon implies a complexity of miRNA-target regulatory network in cellular homeostasis. The dysregulation of miRNA may contribute to numerous disorders, including cancer, immune-related and neurodegenerative diseases [15].

Aberrant expression of miR-196 in various cancers

MiRNAs are frequently found located at the genomic fragile sites or the oncogenic associated regions, which suggests that miRNA molecules

participate in malignant transformation [16, 17]. Accumulating studies have demonstrated that miRNAs can serve as either tumor suppressors or oncogenes [18-20]. Recently, miR-196 has been found aberrantly expressed in many cancers. There are three miR-196 genes in human cells: miR-196a-1, miR-196a-2, and miR-196b. They are all located in the HOX gene clusters and produce two mature miRNA family members: miR-196a and miR-196b [21]. Table 1 shows the miR-196 family genes and their mature sequences. The miR-196a-1 and miR-196a-2 genes are located on chromosomes 17 and 12, respectively [22]. Both of these genes transcribe the same mature miR-196a molecule, which has the sequence 5'-UAGGUA- GUUUCA- UGUUGU- UGGG-3'. The miR-196b gene is located on chromosome 7 and transcribes a mature miR-196b molecule with the sequence 5'-UAGGUA- GUUUC- UGUUGU- UGGG-3' [23]. There is only one nucleotide difference between mature miR-196a and miR-196b, though both possess the same seed region.

Although high similarity exists between miR-196a and miR-196b, these two molecules have a wide range of expression and diverse roles in various types of cancers. For example, miR-196a has been reported to be over-expressed in head-neck [24], oral [25, 26], gastric [27, 28], pancreatic [29, 30], cervical [31, 32], and lung [33] cancers. Similarly, miR-196b has been found to be up-regulated in the cancers of oral [25], gastric [34], colorectal [35, 36], as well as in glioblastoma [37] and in leukemia [23, 38]. The functions of the oncogenic effects of miR-196 family molecules include promoting cell proliferation, migration, invasion and radioresistance [21-34]. However, few reports have indicated that the miR-196 family may act as tumor suppressors. For example, miR-196a suppresses metastasis in melanoma [39] and breast cancers [40], and miR-196b is down-regulated in different types of leukemia cells [41, 42]. Collectively, both miR-196a and miR-196b are frequent aberrations in malignant diseases, and which indicates their close association with disease status.

Table 1. The information of miR-196 family genes and the mature miR-196 molecules.

miRNA gene	Accession No.	Gene location	Stem-loop sequence	Mature miRNA	ID number (Mature miRNA)	Accession No. (Mature miRNA)	Mature miRNA Sequence (Seed#)
hsa-mir-196a-1	MI0000238	Chr17: 48632490-48632559	GUGAAUUAGGUAGUUUCAUGUUGUUGGGC CUGGGUUUCUGAACACAACAACAUAAAC CACCCGAUUCAC	hsa-mir-196a-5p	hsa-miR-196a	MIMAT0000226	<u>UAGGUAGUUUCA</u> UGUUGUUGGG
hsa-mir-196a-2	MI0000279	Chr12: 53991738-53991738	UGCUCGCUCAGCUGAUCUGGGCUUAGGU AGUUUCAUGUUGUUGGGAUUGAGUUUUGA ACUCGGCAACAAGAAACUGCCUGAGUUAC AUCAGUCGGUUUUCGUCGAGGGC	hsa-mir-196a-5p	hsa-miR-196a	MIMAT0000226	<u>UAGGUAGUUUCA</u> UGUUGUUGGG
				hsa-mir-196a-3p	hsa-miR-196a*	MIMAT0004562	<u>CGCAACAAGAA</u> ACUGCCUGAG
hsa-mir-196b	MI0001150	Chr7: 27169480-27169563	ACUGGUCGGUAGUUUAGGUAGUUUCCUGU UGUUGGGAUCCACCUUUCUCGACAGCA CGACACUGCCUACUACUUCAGUUG	hsa-miR-196b-5p	hsa-miR-196b	MIMAT0001080	<u>UAGGUAGUUUCC</u> UGUUGUUGGG
				hsa-miR-196b-3p	hsa-miR-196b*	MIMAT0009201	<u>UCCACAGCACGA</u> CACUGCCUUC

Seed regions are underlined in the mature miRNA sequences.

Table 2. Summary of the target genes and functions of miR-196 family molecules in diverse cancers.

miR-196	Cancers	Family member	Target genes	Function	References
over-expressed	Head-neck cancer	miR-196a	Annexin A1	Proliferation, invasion, radioresistance	[24]
	Oral cancer	miR-196a, miR-196b	NME4	Migration, invasion	[25, 26]
	Esophageal cancer	miR-196a	Annexin A1	Cell growth	[43]
	Gastric cancer	miR-196a, miR-196b	p27 ^{kip1} , HOXA10, Radixin	Proliferation, metastasis	[27, 34, 44]
	Colorectal cancer	miR-196a, miR-196b	HOXA7/B8/C8/D8), FAS	Mobility, apoptosis	[36, 45]
	Pancreatic cancer	miR-196a	NFKBIA	Proliferation, migration	[29, 30]
	Non-small cell lung cancer	miR-196a	HOXA5	Proliferation, invasion	[33]
	Cervical cancer	miR-196a	netrin 4/FOXO1/ p27 ^{kip1}	Proliferation, migration	[31, 32]
	Acute myeloid leukemia	miR-196a, miR-196b	ERG	Hematopoiesis	[23, 38]
	Glioblastoma	miR-196b	--	Proliferation	[37]
under-expressed	Acute lymphoblastic leukemia	miR-196b	c-Myc	---	[38]
	Melanoma	miR-196a	HOXB7/BMP4/HOXC8	Suppression of migration and invasion	[39, 48]
	Chronic myeloid leukemia	miR-196b	BCR-ABL1/HOXA9	---	[41]

Numerous functions of miR-196 in different cancers may be due to various gene targets

Because miRNAs function as gene regulators, identifying miRNA targets allows delineation of the specific function of a miRNA. Several computational software packages have been developed to predict potential miRNA targets by aligning the conserved seed region of a miRNA to the complementary sequences on the 3'-UTR region of a mRNA [43]. Many experiments have further confirmed the regulatory axis. Table 2 summarizes the expression, target genes, and functions of miR-196 family molecules in diverse cancers. The oncogenic functions of these molecules have been found to target diverse tumor suppressors in specific types of cancer tissues. For example, in oral cancer, both miR-196a and miR-196b down-regulate the expression of metastasis suppressor NME4 to accelerate cell migration and invasion [25]. In esophageal and head-neck cancers, miR-196a targets Annexin A1 to promote cell growth, mobility and radioresistance [24, 44]. In gastric cancer, miR-196a down-regulates p27^{kip1}, a cell cycle G1 check-point regulator, to promote cell proliferation [27]. Also in gastric cancer, both miR-196a and miR-196b promote cell metastasis via suppression of radixin [45]. In pancreatic cancer, miR-196a targets NFκB-1α to promote cancer progression [29]. In colorectal cancer, miR-196a restrict the expression of HoxA7, HoxB8, HoxC8 and HoxD8 genes, leading to the activation of the AKT pathway and increase cell mobility [46]. A high level of miR-196b in colorectal cancer also repressed FAS expression to modulate cell apoptosis [36]. In cervical cancer, miR-196a inhibits p27^{kip1}, FOXO1 or netrin 4 to facilitate cell proliferation or migration [31, 32]. Nevertheless, few studies have reported on the function of the miR-196 family as a tumor suppressor by targeting oncogenes. For example, in melanoma and breast cancer, miR-196a suppresses HOXC8, a transcription factor,

to inhibit cell growth, invasion and metastasis [47, 48]. In chronic myeloid leukemia, miR-196b is down-regulated in cells and enhances the functions of oncogenes BCR-ABL1 and HOXA9 [41].

Aside from the miRNA-downstream target gene axis, the upstream regulatory mechanism of miR-196 has also been reported, mainly through epigenetic associated mechanism [49]. The CpG islands in the miR-196b promoter showed more methylation in chronic myeloid leukemia patients than healthy individuals [41]. In gastric cancer, miR-196b has been found regulated by Est2 through an epigenetic pathway [50, 51]. Furthermore, miR-196a2 gene possesses single nucleotide polymorphism in many cancers, including in breast, head-neck, lung and oral cancers, which may result to target binding deficit and increasing cancer susceptibility [26, 52-58]. Taking together, miR-196 family molecules play important roles in homeostasis, and dysregulation of this family closely associated with various malignancy.

Over-expression of miR-196 is commonly found in digestive tract cancer tissues and associated with aggressive clinicopathological status

Although vary in level, miR-196 family molecules are consistently found over-expressed in digestive tract cancer tissues, which comprise of mouth, esophagus, stomach, small intestine, and large intestine. Table 3 summarizes the clinicopathological association of miR-196 family molecules in digestive tract cancers. On the differentiation of normal and cancer tissues, both miR-196a and miR-196b are significantly elevated in oral cancer tissues compared to the adjacent normal counterparts [25], indicating that miR-196 may be a putative diagnostic marker. Consistently, miR-196a is up-regulated in laryngeal cancer and able to discriminate normal and cancer tissues [59]. In esophagus, miR-196a shows correlative expression during cancer progression, as gradually higher from normal tissue, metaplasia, dysplasia, to

invasive adenocarcinoma [60]. In addition to the diagnostic potential, miR-196 family molecules have also been reported associated with clinicopathological status of the digestive tract cancers, which implies the potential in using of these molecules in prognostic application. For examples, both miR-196a and miR-196b are up-regulated in oral cancer tissues and associated with lymph node metastasis [25]. The high level of miR-196a in head-neck cancer tissue also contributes to radioresistance [24]. In gastric cancer, miR-196a or miR-196b is linked to advanced pathologic stage, cancer recurrence and shorter survival [27, 34, 45, 61]. Concordantly, both miR-196a and miR-196b in colorectal cancer shows cooperatively correlated with aggressive disease and unfavorable to treatment outcome [35]. Taken together, miR196 family molecules in digestive tract cancers may serve as biomarkers for clinical applications in diagnosis, prognosis, or prediction of treatment efficacy.

Circulating miR-196 may serve as a novel cancer biomarker for digestive tract cancers

Detecting biomolecules in body fluids, including peripheral blood, urine or saliva, provides a less invasive process to facilitate clinical applications. Recently, many reports have revealed that miRNAs can be exosomal released from tumor cells, stably expressed in circulation, which may be suitable to be utilized as promising biomarkers [62-65]. Regarding miR-196 family, several studies have shown that miR-196 molecules are detectable in the peripheral blood from digestive tract cancer patients and

associated with clinical significance. For example, miR-196a and miR-196b are significantly elevated in the sera of patients with chronic pancreatitis or pancreatic cancer compared to those from normal individuals [66, 67]. Concordantly, the combined determination of miR-196a and miR-196b produces high sensitivity and specificity for the early detection of pre-malignant or malignant oral diseases [68]. Circulating miR-196a also showed high potential to be a prognostic marker because it correlated with patient survival in recurrence status of gastric cancer [28] or in pancreatic cancer [67].

As stated above, miR-196 is elevated in the blood of patients with several types of digestive tract cancers. However, these results were obtained from various investigators who used different assay protocols which may lead to possible bias. To assess the diagnostic potential, we compared the miR-196b level in the plasma from healthy individuals and from patients with several types of cancers, including nasopharyngeal, oral, esophageal, gastric, colorectal and lung cancers. The results are shown in Figure 1. MiR-196b was substantial increased in digestive tract cancer patients, with an average elevation of 3.7- to 25.6-fold ($P < 0.001$ for all). The detection power of miR-196b for each cancer is summarized in Table 4 (Youden Index [69]). Such high ability to discriminate between healthy individuals and cancer patients indicates that miR-196b may serve as a powerful circulating tumor marker for the detection of digestive tract cancers.

Table 3. Summary of the clinicopathological significance of miR-196 family molecules in digestive tract cancers.

Cancers.	Family member	Diagnostic potential	Clinicopathological or prognostic association	References
Oral cancer **	miR-196a, miR-196b	Differentiate normal, pre-cancer and cancer	Associated with lymph node metastasis	[25][68]
Head-neck cancer #	miR-196a	Differentiate normal and cancer	Associated with radioresistance	[59, 24]
Esophageal cancer #	miR-196a	Differentiate normal, pre-cancer and cancer	--	[60]
Gastric cancer **	miR-196a, miR-196b	--	Associated with recurrence and shorter survival	[27,28,34,45,61]
Colorectal cancer #	miR-196a, miR-196b	--	Associated with shorter survival	[35]
Pancreatic cancer*	miR-196a	Differentiate normal and cancer	Associated with recurrence and shorter survival	[66, 67]

#Determination of miR-196 in tissue specimens, * Determination of miR-196 in blood specimens.

Table 4. The statistical result for each cancer (Mann-Whitney test).

Plasma from	Sample number	Relative value (Mean)	SEM	P-value (w/normal)	Area under ROC curve (AUC)	(%) Sensitivity/ (%) specificity**
Normal	30	6.1	1.345	--	--	--
Lung cancer	10	4.5	1.398	N.S.	0.552	30/97
Oral cancer	20	73.5	11.00	<0.0001	0.990	100/94
Nasopharyngeal carcinoma	9	67.7	11.68	<0.0001	0.996	100/97
Esophageal carcinoma	10	156.4	55.74	0.0008	0.858	70/100
Gastric cancer	20	78.0	24.97	<0.0001	0.924	95/77
Colorectal cancer	28	22.6	3.806	<0.0001	0.821	82/77

N.S.: No statistical significance by Mann-Whitney test.

**.: The optimal cutoff threshold for diagnosis was obtained by applying the Youden's Index (sensitivity + specificity -1 is maximal) [69].

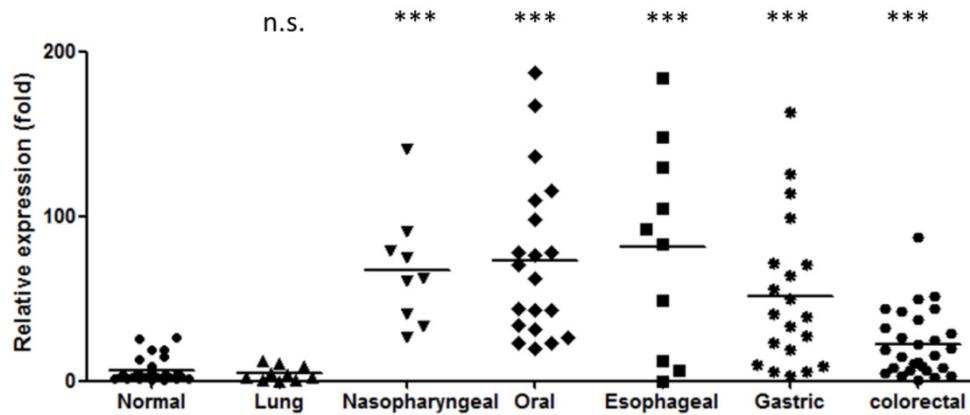


Figure 1. Elevation of miR-196b levels in the plasma of patients with digestive tract cancer. (n.s.: no statistical significance compared to normal group Mann-Whitney test, ***: $P < 0.001$ compared to normal group Mann-Whitney test).

Concluding remarks

MiR-196 offers a great potential as a biomarker for cancer detection, diagnosis, prognosis, and therapeutic assessment via both tumor tissues and circulating specimens. It will be imperative to carry out prospective trials in well-defined, large cohort studies to validate its clinical significance. Determining the miR-196 level by using a minimally invasive method may be developed and applied in personalized cancer medicine.

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Competing interests

The authors have declared that no competing interest exists.

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