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The role of microRNAs in gastrointestinal cancers

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MicroRNAs (miRNAs) are small noncoding RNAs that function as endogenous silencers of numerous target genes. Hundreds of human miRNAs have been identified in the human genome, and they are expressed in a tissue-specific manner and play important roles in cell proliferation, apoptosis, and differentiation. Links between miRNAs and human diseases are increasingly apparent, and aberrant expression of miRNAs may contribute to the development and progression of human malignancies. Recent studies have shown that some miRNAs play roles as tumor suppressors or oncogenes in gastrointestinal cancers. miRNA expression is regulated by different mechanisms including transcription factor binding, epigenetic alterations, and chromosomal abnormalities. miRNA expression profiling may be a powerful clinical tool for cancer diagnosis, and regulation of miRNA expression could be a novel strategy for the chemoprevention of human gastrointestinal cancers. In this article, the biological importance of miRNAs in human gastrointestinal cancers is summarized.

Key words: microRNA, gastrointestinal cancer, epigenetics, *Helicobacter pylori*, cyclooxygenase (COX)

The biogenesis of microRNAs (miRNAs)

MicroRNAs (miRNAs) are ~22-nucleotide (nt), noncoding RNAs that can posttranscriptionally downregulate various target genes. Currently, ~700 human miRNAs have been identified in the human genome, and each miRNA potentially controls hundreds of gene targets. miRNAs are expressed in a tissue-specific manner and play important roles in cell proliferation, apoptosis, and differentiation.[1] Moreover, recent studies have shown a connection between aberrant expression of miRNAs and the development of cancer.[2]

In animals, miRNA genes are generally transcribed by RNA polymerase II (pol II) to form primary transcripts (pri-miRNAs). Pol II-transcribed pri-miRNAs are capped with 7-methylguanosine and polyadenylated. The nuclear RNase III enzyme Drosha and its cofactor Pasha (also known as DGCR8) process pri-miRNAs into ~60 nt precursor miRNAs (pre-miRNAs), which form an imperfect stem-loop structure. Pre-miRNAs are transported into the cytoplasm by the RAN GTP-dependent transporter exportin 5 and are subsequently cleaved by the cytoplasmic RNase III enzyme Dicer into mature miRNAs, which are then loaded into the RNA-induced silencing complex (RISC). The miRNA/RISC complex downregulates specific gene products by translational repression via binding to partially complementary sequences in the 3′-untranslated regions (3′-UTRs) of the target mRNAs or by directing mRNA degradation via binding to perfectly complementary sequences (Fig. 1). Although much has been learned about the biogenesis of miRNAs, further studies are necessary to verify the mechanistic details in biological function of miRNAs.

miRNA target prediction

Identification of miRNA target genes is critical to determining miRNA function. Recent studies have indicated that a single miRNA may regulate more than 200 target genes. This target pool is enriched in genes involved in transcriptional regulation and signal transduction. There are several databases of human miRNA target predictions using different algorithms, such as miRanda, miRBase, PicTar, and TargetScan.[3–6] It is generally believed that conserved perfect 6- to 8-base-pair (bp) matches between the 5′-end of the mature miRNA and

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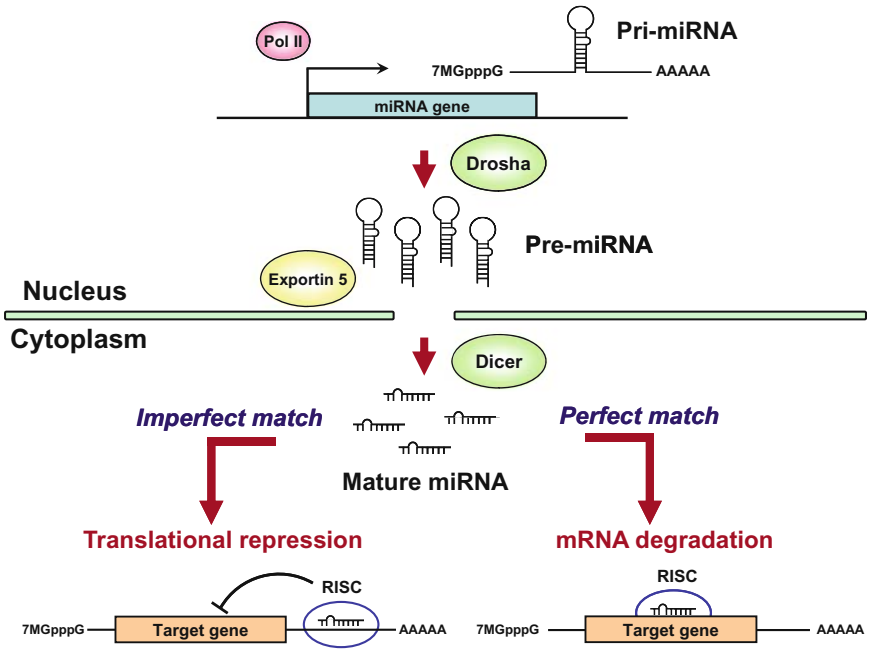

Fig. 1. The biogenesis of microRNAs (miRNAs). miRNA genes are generally transcribed by RNA pol II to form pri-miRNAs, which are capped with 7-methylguanosine (*7MGpppG*) and polyadenylated (*AAAAA*). Drosha and its co-factor Pasha process pri-miRNAs into pre-miRNAs. Pre-miRNAs are transported into the cytoplasm by the RAN GTP-dependent transporter exportin 5 and are subsequently cleaved by Dicer into mature miRNAs. Mature miRNAs are then loaded into RISC, where miRNAs downregulate specific gene products by translational repression via binding to partially complementary sequences in the 3′-UTR of the target mRNAs or by directing mRNA degradation via binding to perfectly complementary sequences. *ORF*, open reading frame

the 3′-UTR of the predicted target mRNA (called "seed" matches) are the most reliable way to determine miRNA targets. Experimental confirmation using a protein expression assay and a luciferase reporter assay for the miRNA target is necessary to accurately identify target genes of miRNAs.

miRNAs play roles as tumor suppressors or oncogenes in gastrointestinal malignancies

The finding that predicted targets of miRNAs are enriched for genes involved in transcriptional regulation, cell proliferation, and apoptosis implies that aberrant expression of miRNAs might contribute to the development and progression of human malignancy. Recent studies have shown a distinct connection between aberrant expression of miRNAs and human carcinogenesis.[2] Recent studies have shown that some miRNAs are downregulated in gastrointestinal cancers, which suggests that they may function as tumor suppressors (Table 1). *miR-15b* and *miR-16*, which are downregulated in human gastric cancer cells, play a role in the development of multidrug resistance (MDR) by modulation of apoptosis via targeting *BCL2*.[7] *miR-34a*, which is decreased in human colon cancers, functions as a tumor suppressor and induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells.[8] *miR-143* and *miR-145* are downregulated in colorectal cancer.[9] Akao et al. showed that in colorectal cancer, *miR-143* targets *ERK5*, an important member of signal transduction pathways involved in proliferation and cell survival.[10] This finding suggests that *miR-143* is a tumor suppressor whose downregulation may contribute to an oncogenic phenotype.

On the other hand, some miRNA genes are overexpressed in gastrointestinal cancers, indicating that they may have roles as oncogenes and accelerate the development of gastrointestinal cancer (see Table 1). *miR-155* and its host gene *BIC* are highly expressed in several types of B-cell lymphoma.[11] A recent study has shown that *miR-155* is overexpressed in several types of human solid tumors including colon cancer.[12] Gironella et al. have demonstrated that the expression of *tumor protein 53-induced nuclear protein 1* (*TP53INP1*), a pro-apoptotic stress-induced p53 target gene, is repressed by the oncogenic miRNA *miR-155*, which is overexpressed in pancreatic ductal adenocarcinoma cells, and suggested that *TP53INP1* is a target of *miR-155*.[13] The *miR-17-92* cluster, which is located on chromosome 13q31, is activated by the oncogene c-*Myc*, which is an important regulator of cell growth and is often mutated or amplified in human cancers. The *miR-17-92* cluster is highly expressed in various human malignancies including colon cancer.[12,14] c-*Myc* upregulates the transcription factor *E2F1*, and O'Donnell et al. found that two miRNAs in the *miR-17-92* cluster, *miR-17-5p* and *miR-20a*, target *E2F1*.[15] Their work shows that there is a negative feedback loop involving c-*Myc*, *E2F1*, and *miR-17-5p* and *miR-20a*. *miR-20a* also targets *transforming growth factor-beta receptor 2* (*TGFBR2*), which is a key mediator of TGF-β signaling and strongly implicated in human carcinogenesis.[12] *miR-21* is upregulated in various human malignancies including stomach and colon cancers.[12] Moreover, *miR-21* is highly overexpressed in human cholangiocarcinoma and modulates

Table 1. MicroRNAs (miRNAs) as tumor suppressors or oncogenes in gastrointestinal cancers

miRNA	Roles in gastrointestinal cancers	Target genes	References
Potential tumor suppressor miRNAs:			
miR-15b, miR-16	*miR-15b* and *miR-16* play a role in the development of MDR in gastric cancer cells by modulation of apoptosis via targeting *BCL2*	*BCL2*	7
miR-34a	*miR-34a* functions as a tumor suppressor and induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells	E2F pathway	8
miR -143, miR-145	*miR-143* and *miR-145* are downregulated in colorectal cancer	*ERK5*	9, 10
Potential oncogenic miRNAs:			
miR-17-92 cluster	*miR-17-92* cluster is overexpressed in various human malignancies, including colon cancer	*E2F1* (*miR-17-5p, miR-20a*) *TGFBR2* (*miR-20a*)	12, 14, 15
miR-21	*miR-21* is upregulated in various human malignancies including cholangiocarcinoma, and gastric and colon cancers	*PTEN*	12, 16
miR-106a	*miR-106a* is upregulated in colon cancer	*RB-1*	12
miR-106b-25 cluster	*miR-106b-25* cluster, which is upregulated in human gastric cancers and activated by E2F1, impairs the TGF-β tumor suppressor pathway	*p21* *Bim* *E2F1*	17
miR-155	*miR-155* is overexpressed in various human malignancies, including B-cell lymphoma and colon cancer	*TP53INP1*	12, 13

gemcitabine-induced apoptosis by directly altering *PTEN*, which regulates cellular proliferation, growth, and apoptosis as a tumor suppressor gene.[16] The *miR-106b-25* cluster, which is upregulated in human gastric cancers and activated by E2F1, impairs the TGF-β tumor suppressor pathway by interfering with the expression of *p21* and *Bim*.[17] *miR-106a* is also upregulated in colon cancer and targets *RB-1*.[12] These findings indicate that miRNAs have critical roles in the mechanism underlying human carcinogenesis and that aberrant expression of miRNAs may contribute to the initiation and progression of human gastrointestinal cancers.

Regulatory mechanisms of miRNA expression

Because miRNAs can have large-scale effects through regulation of a variety of genes during mammalian development and carcinogenesis, understanding the regulatory mechanisms controlling miRNA expression is quite important. However, the regulation of miRNA expression is not fully understood. There are several reports describing that transcription factors bind to the promoter regions of specific miRNA genes and activate the transcription of pri-miRNAs, resulting in increased expression of mature miRNAs. As we mentioned earlier, c-Myc binds to the regulatory region of the *miR-17-92* cluster, and increased expression of *c-Myc* leads to the activation of the miRNAs in the cluster. On the other hand, numerous human miRNAs have been shown to reside within the intronic regions of either coding or noncoding transcription units. It is believed that intronic miRNAs are coordinately expressed with their host gene mRNA and that the expression of intronic miRNAs depends on the regulation of their host genes.[18] The location of miRNA genes is also an important factor for the regulation of miRNA expression. Many miRNAs are located at cancer-associated genomic regions that are frequently involved in chromosomal abnormalities such as loss of heterozygosity (LOH), amplification and breakpoints.[19] Chromosomal abnormalities during carcinogenesis could lead to the widespread differential expression of miRNAs in human cancer cells.

Epigenetic changes such as DNA methylation and histone modification play critical roles in chromatin remodeling and regulation of gene expression in mammalian development and in human diseases. Many miRNAs are expressed in a tissue- and tumor-specific manner, implying that some miRNAs are subject to epigenetic control. We have recently shown that *miR-127*, which is embedded in a CpG island, is strongly induced by treatment with the DNA demethylating agent 5-aza-2′-deoxycytidine (5-Aza-CdR) and the histone deacetylase (HDAC) inhibitor 4-phenylbutyric acid (PBA), indicating that some miRNA genes are controlled by epigenetic alterations in their promoter regions and can be activated by chromatin-modifying drugs.[20]

The miRNA profile as a tool for diagnosis and prognosis of cancer

Lu et al. reported that miRNA expression profiles can be used to classify the developmental lineages and differentiation stages of tumors.[21] Interestingly, miRNA expression profiles are more accurate for tumor classification than conventional mRNA profiles.[21] Furthermore, recent studies demonstrated that miRNA expression signatures are associated with prognostic factors and disease progression in chronic lymphocytic leukemia and lung cancer.[22,23] Yanaihara et al. showed that low *let-7a-2* expression is correlated with poor survival of patients with lung adenocarcinoma, indicating that *let-7* may have a role as a tumor suppressor. On the other hand, overexpression of *miR-155*, which is a potential oncogenic miRNA, correlates with poor survival in lung cancers.[23] Schetter et al. have shown that high *miR-21* expression is associated with poor survival and poor therapeutic outcome in colon cancer.[24] These findings indicate that the miRNA expression profile might be a powerful clinical tool for the diagnosis and prognosis of patients with cancer, especially in a case that is difficult to diagnose pathologically.

Role of miRNAs in the chemoprevention of gastrointestinal cancers

The distinct connection between aberrant expression of miRNAs and the initiation and progression of cancer suggests that miRNAs could be novel therapeutic targets.

Cyclooxygenase (COX) is a critical enzyme involved in prostaglandin production and has two isoforms. COX-1 is constitutively expressed in normal tissues, whereas COX-2 is overexpressed in various human malignancies. Therefore, selective COX-2 inhibitors have been proposed to be a potential drug for the chemoprevention of gastrointestinal cancers. Recent clinical trials revealed that the selective COX-2 inhibitor celecoxib is an effective agent for the chemoprevention of colorectal adenomas, although, because of potential cardiovascular events, it cannot be routinely recommended.[25,26] However, the molecular mechanisms underlying the chemopreventive effects of selective COX-2 inhibitors are not fully understood. To investigate the roles of miRNAs in anticancer effects of selective COX-2 inhibitors, we have recently analyzed the miRNA expression profile in human gastric cancer cells treated with celecoxib. Mott et al. recently reported that *miR-29c* is an endogenous regulator of Mcl-1, an antiapoptotic Bcl-2 family member.[27] We have found that *miR-29c*, which is significantly upregulated after the treatment, induces apoptosis by suppressing Mcl-1 target oncogene (unpublished data). This pathway could be one of the mechanisms of the chemopreventive effects of selective COX-2 inhibitors.

Epigenetic therapy with chromatin-modifying drugs has clinical promise for the chemoprevention of cancer. DNA methylation inhibitor 5-Aza-CdR has been widely studied and was recently approved by the FDA for the treatment of myelodysplastic syndrome (MDS). Many HDAC inhibitors are also in clinical trials.[28] We have recently proposed that a novel beneficial effect of epigenetic therapy is the downregulation of oncogenes via the activation of tumor suppressor miRNAs.[29]

Perspective in miRNAs and *Helicobacter pylori*-associated gastric cancer

In spite of the decline in gastric cancer incidence in Western countries, gastric cancer is the fourth most common cancer in the world, as the incidence of this disease remains very high in East Asia and in South America.[30] Many researches now focus on the effect of *Helicobacter pylori* eradication[31] on gastric cancer prevention in the general population as well as for patients with preneoplastic lesions such as chronic atrophic gastritis and intestinal metaplasia. Several randomized placebo-controlled studies have reported that *H. pylori* eradication could, to some extent, induce a restoration of atrophy.[32–36] The effect of bacterial eradications on gastric cancer prevention is, however, less evident.[33,35,37] In all these studies,[32–37] there was no significant difference between the *H. pylori* eradication and placebo groups in terms of the incidence of gastric cancer in the first 4–12 years after treatment. The outstanding observation in all these studies, however, was that those gastric cancers that developed after *H. pylori* eradication were in particular confined to those subjects who already had atrophic gastritis and intestinal metaplasia, suggesting that the major cancer-preventive effect of *H. pylori* eradication is to be expected in subjects without such precancerous conditions. In other words, cohorts with preneoplastic lesions may already have passed the point of no return and need to be further treated in addition to *H. pylori* eradication. Maekita et al. have demonstrated that *H. pylori* infection potently induces methylation of promoter regions of some genes.[38,39] Long-term colonization of *H. pylori* might induce epigenetic modification of gastric mucosal genes, including promoter of tumor suppressor miRNAs, which cannot be completely reversed only by bacterial eradication. Epigenetic therapy on the severe atrophic or metaplastic gastric mucosa after *H. pylori* eradication might be a possible option for gastric cancer prevention.

As the relationship between miRNAs and cancer has only just begun to be understood and the number of

identified miRNA genes is increasing, there could be a large number of therapeutic targets of gastrointestinal cancers. Further studies are necessary to investigate whether miRNA-mediated therapy is an effective strategy for the chemoprevention of human gastrointestinal cancers.

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