Two common gastrointestinal cancers, namely, gastric and colorectal cancers, cause high mortality and morbidity. The development of gastrointestinal cancers usually follows stepwise processes with recognizable pre-neoplastic changes. A class of noncoding RNA known as microRNA (miRNA) is increasingly recognized to play pleiotropic functions in the multistep development of gastrointestinal cancers. Abnormal patterns of miRNA expression in gastric and colorectal cancers have been widely reported. These dysregulated miRNAs function as novel proto-oncogenes and tumor-suppressor genes by controlling cellular malignant phenotypes, including unchecked cell proliferation, resistance to apoptosis, enhanced invasiveness and metastasis, and angiogenesis. Moreover, certain polymorphisms in miRNA genes or miRNA-binding sites are associated with disease risks whereas detection of circulating or fecal miRNAs may facilitate early diagnosis. The prognostic functions of a number of dysregulated miRNAs in gastrointestinal cancers have also been established. Delineating the pathophysiological basis of miRNA dysregulation will further our understanding of the pathogenesis of these two potentially fatal diseases. Such efforts will also result in the development of miRNA-based biomarkers and therapeutics for the risk stratification, diagnosis, prognostication, and treatment of gastrointestinal cancers. (Intest Res 2012;10:324-331)

Key Words: Colorectal cancer; Gastric cancer; MicroRNA; Signaling pathway; Prognosis

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protein kinase cascade, the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) cascade, p53 signaling, nuclear factor κ B signaling, Wnt/β-catenin signaling, transforming growth factor (TGF) β signaling, etc. These signaling pathways regulate cellular processes related to tumorigenesis, including cell proliferation, apoptosis, cellular motility and invasiveness, angiogenesis, inflammation, and multi-drug resistance. Emerging evidence supports that microRNAs (miRNAs), a class of non-coding RNA of 18-25 nucleotides in length that mediates their biological function through repression of target gene expression, are essential components of intracellular signaling networks and their dysregulation is involved in the pathogenesis of malignant diseases, including gastric and colorectal cancers. In this review, the contribution of miRNAs to the acquisition of malignant phenotypes in gastric and colon cancer cells as well as their potential clinical applications of novel diagnostic and prognostic markers will be discussed.

BIOGENESIS AND MECHANISTIC ACTION OF miRNA

While most miRNA genes function as independent transcription units, up to a quarter of them are intronic and share promoters and regulatory elements with their host genes. Transcribed by RNA polymerase II, miRNA gene encodes the primary miRNA (pri-miRNA) consisting of a 5’ cap, at least one ~70-nucleotide stem-loop structure and a 3’ poly(A) tail. Polycistronic pri-miRNA may harbor up to seven stem-loop structures that give rise to different mature miRNAs. After transcription, pri-miRNA is processed into precursor miRNA by DROSHA-mediated removal of 5’ cap, 3’ poly(A) tail and sequences flanking the hairpin structure. Nuclear precursor-miRNA is then exported by exportin-5 to the cytoplasm. Precursor-miRNA is further processed in the cytoplasm by the endoribonuclease DICER to produce a pair of short double-stranded RNA fragments. The mature miRNA strand is then incorporated into the RNA-induced silencing complex (RISC) (Fig. 1). In partnership with RISC, the mature miRNA strand binds to the 3’ untranslated regions (UTRs) of its target messenger RNAs (mRNAs) and represses their expression through the following mechanisms: (1) argonaute-mediated cleavage of target mRNAs; (2) cap-dependent inhibition of translation initiation; (3) sequestration of eukaryotic translation initiation factor-6 by RISC; (4) induction of nascent protein degradation; (5) ribosomal drop-off; (6) induction of mRNA deadenylation; (7) preventing the interaction between poly(A)-binding proteins and eukaryotic translation initiation factor-4G.

Although miRNA only accounts for 1-3% of the human genome, it has been estimated that up to 30% of
The biogenesis of miRNA has been dysregulated in gastric and colorectal cancer. For example, it has been reported that the mRNA and protein expression of Dicer1 are significantly reduced during the disease progression of gastric cancer.\(^1\) Ago2 and TNRC6A, both of which are components of RISC, are also mutated and downregulated in a subset of gastric and colon cancer patients with microsatellite instability.\(^2\) On the contrary, another RISC component known as Sord1 is upregulated in aberrant crypt foci and colon cancer.\(^3\) The impact of these dysregulations to the biogenesis of miRNA in gastrointestinal cancers, however, warrants further comprehensive analysis.

### DYSREGULATION OF miRNA IN GASTROINTESTINAL CANCERS

Overexpression or downregulation of numerous miRNAs have been documented in gastric and colorectal cancers. Dysregulated miRNAs in gastrointestinal cancers reported by three or more independent studies together with their mRNA targets are listed in Table 1.\(^4\-\!^8\)

### REGULATION OF ONCOGENIC PHENOTYPES BY miRNA

#### 1. Cell Proliferation

Unchecked cell proliferation is a common feature of malignant diseases. Progression through the cell cycle is orchestrated by the temporally coordinated expression and interaction among different cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors. In particular, two families of CDK inhibitors, namely, p16 family (p15, p16, p18 and p19) and p21 family (p21, p27, p28 and p57), play essential roles in the negative regulation of cell cycle. The former mainly inhibits G1-S transition while the latter inhibits all phases of the cell cycle. A number of miRNAs have been shown to alter cell proliferation in gastric and colorectal cancers through interacting with important components in the cell cycle and mitogenic pathways. For instance, miR-106b and miR-93, both of which are upregulated in gastric cancer tissues, have been shown to directly target the CDK inhibitor p21 to promote cell cycle progression.\(^9\) Two upregulated miRNAs, namely, miR-222 and miR-221, also repress the expression of p27 and p57 in gastric cancer tissues. Concordantly, ectopic expression of miR-221 and miR-222 enhances the growth of gastric cancer xenograft in nude mice.\(^10\) In colorectal cancer, let-7a and miR-143 remarkably reduce the expression of K-Ras, an oncogenic small GTPase whose mutation have been detected in 27-43% of patients with colorectal cancer, and thereby inhibiting cell proliferation and/or colony-forming ability. An inverse correlation between K-Ras protein and miR-143 expression has been demonstrated in human colon cancer tissues.\(^11\)

### Table 1. List of Dysregulated miRNAs in Gastric and Colorectal Cancers Reported by Three or More Independent Studies

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Dysregulation</th>
<th>Target gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7a</td>
<td>Downregulated</td>
<td>HMGA2</td>
</tr>
<tr>
<td>miR-17-5p</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-18a</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-19a</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-20a</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-21</td>
<td>Upregulated</td>
<td>PDCD4, RECK</td>
</tr>
<tr>
<td>miR-25</td>
<td>Upregulated</td>
<td>Bim, p57</td>
</tr>
<tr>
<td>miR-31</td>
<td>Downregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-92</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-93</td>
<td>Upregulated</td>
<td>E2F-1, p21</td>
</tr>
<tr>
<td>miR-106a</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-106b</td>
<td>Upregulated</td>
<td>E2F-1, p21</td>
</tr>
<tr>
<td>miR-218</td>
<td>Downregulated</td>
<td>ECOP, Robo1</td>
</tr>
<tr>
<td>miR-221</td>
<td>Upregulated</td>
<td>p27, p57</td>
</tr>
<tr>
<td>miR-223</td>
<td>Upregulated</td>
<td>N.D.</td>
</tr>
<tr>
<td>miR-375</td>
<td>Downregulated</td>
<td>PDK1, 14-3-3zeta</td>
</tr>
</tbody>
</table>

Gastric cancer

| let-7a | Downregulated | c-Myc, DLD-1, Ras |
| miR-1  | Downregulated | N.D.              |
| miR-17 | Upregulated   | E2F1              |
| miR-18a| Upregulated   | N.D.              |
| miR-19a| Upregulated   | N.D.              |
| miR-19b| Upregulated   | N.D.              |
| miR-20a| Upregulated   | N.D.              |
| miR-21 | Upregulated   | Cdc25A, Pdcd4, PTEN, Sprouty2 |
| miR-25 | Upregulated   | N.D.              |
| miR-29a| Upregulated   | N.D.              |
| miR-30a| Downregulated | N.D.              |
| miR-31 | Upregulated   | FIH-1             |
| miR-92a| Upregulated   | N.D.              |
| miR-96 | Upregulated   | N.D.              |
| miR-106a| Upregulated  | E2F1              |
| miR-106b| Upregulated  | N.D.              |
| miR-133a| Downregulated| N.D.              |
| miR-135b| Upregulated  | APC               |
| miR-137| Downregulated | Cdc42, LSD-1     |
| miR-143| Downregulated | Ras, Erk5, DNMT3A |
| miR-145| Downregulated | IRS1, STAT1, YES, FLI1 |
| miR-181b| Upregulated | CYLD             |
| miR-183| Upregulated   | Sox2, Klf4        |
| miR-191| Upregulated   | N.D.              |
| miR-195| Downregulated | Bcl-2             |
| miR-203| Upregulated   | Sox2, Klf4        |
| miR-224| Upregulated   | N.D.              |
| miR-378| Downregulated | N.D.              |

Colorectal cancer

N.D., not determined.

Revised table from Carcinogenesis 2011;32:247-253 with permission.
24. miR-192 and miR-215 also target dihydrofolate reductase, an S-phase-specific enzyme that produces tetrahydrofolate for the synthesis of purine and thymidylate during cell proliferation, to induce cell cycle arrest. 40-42 miR-34a and miR-675 have been shown to alter colon cancer cell proliferation through regulation of the E2F transcription factor family. The transcriptional activity of E2F is required for the induction of genes, including dihydrofolate reductase, that are essential for DNA synthesis. 42 miR-143, a frequently downregulated miRNA in cancer, also targets insulin receptor substrate 1, an upstream signal mediators of the mitogenic Ras/Raf/mitogen-activated protein kinase and the pro-survival PI3K/Akt cascades. 43 These findings indicate that miRNAs can regulate cell proliferation in both positive and negative manners via interactions with different components in the cell cycle and mitogenic signaling pathways during gastrointestinal tumorigenesis.

2. Apoptosis

Loss of balance between cell proliferation and cell death is central to tumorigenesis. In fact, evasion of apoptosis has been designated as one of the six major hallmarks of cancer. The Bcl-2 family plays an essential role in the regulation of apoptosis. The pro-apoptotic (Bax, Bak, Bik, Bin, Bad, Bid, HRK, NOXA, PUMA, and BNIP3) and anti-apoptotic (Bcl-2, Bcl-xL, Bcl-w, A1, and McI-1) members of the Bcl-2 family govern cell death by controlling mitochondrial membrane permeability, cytochrome c release, and caspase-9 activation. 2 In this respect, miRNA dysregulation has been shown to regulate apoptosis in gastrointestinal cancers through targeting the Bcl-2 family members. In gastric cancer, miR-106b and miR-93 have been shown to abolish TGFβ-induced apoptosis by repressing the expression of Bim, which is a pro-apoptotic member of the Bcl-2 family. 29 miR-15b, miR-16, miR-34, and miR-181b also directly target the antiapoptotic protein Bcl-2 and positively regulate apoptosis in gastric cancer cells. 44,45 In colorectal cancer, miR-195 targets Bcl-2 and induces apoptosis in cancer cell lines. 46 miR-143 also reduces colon cancer cell viability and enhances 5-fluorouracil-induced cell death through downregulation of Bcl-2. 46 In addition, miR-34a has been shown to promote apoptosis through induction of PUMA via the sirtuin 1-p53 cascade in colon cancer cells. 49 Other non-Bcl-2 family components of the apoptotic circuit have also been reported to be targeted by miRNAs. For example, miR-133 targets c-Met, the receptor for hepatocyte growth factor, to induce apoptosis in colon cancer cells. 50 miR-145 also represses DNA fragmentation factor-45, a substrate of caspase-3, to mediate staurosporin-induced apoptosis. 51 All these findings suggest that regulation of the Bcl-2 family and other key members in the apoptotic cascade is central to the modulatory effects of miRNAs on cell death.

3. Metastasis

Metastasis contributes to the high mortality of cancerous diseases. To metastasize, cancer cells must break apart from the primary tumor and degrade the surrounding extracellular matrix. Afterwards, these cells have to enter lymphatic or blood vessels where they must subvert the apoptotic program that is often activated upon detachment of epithelial cells from the substratum and neighboring cells. Eventually, these cells must acquire an ability to proliferate in the new microenvironment at the secondary site. Recent data indicates that miRNA dysregulation might promote the acquisition of these phenotypes in gastric and colon cancer cells. For instance, enforced expression of miR-21 has been shown to increase the invasiveness of cultured gastric cancer cells through repression of RECK, a tumor-suppressor that inhibits tumor metastasis and angiogenesis through modulating MMP9, MMP2 and MMP14 in which enhanced MMP activity is required for the digestion of extracellular matrix. 52 Reduced expression of miR-218 is also involved in gastric cancer metastasis through modulating the Slit/Robo1 signaling, which is a major axon-guidance signaling axis. While the principle functions or roles of axon-guidance molecules in carcinogenesis have not been well established, emerging evidence have hinted at their involvement in the development of gastrointestinal cancers in which a repertoire of axon-guidance molecules (e.g., netrin-1, UNC5A, NEO1, RGMA) have been shown to be dysregulated. 53 In colorectal cancer, miR-196a promotes the migration and invasiveness of cancer cells through direct targeting of HoxA7, HoxB8, HoxC8 and HoxD8, all of which belong to the Hox protein family that is known to be activated during early embryonic development and malignant diseases. 54 miR-196a also concomitantly increased phosphorylation levels of Akt that enhances cellular invasiveness. In addition to miR-196a, miR-21 and miR-141 have been reported to promote the motility and invasiveness of colon cancer cells through targeting PDCD4 and SIP1, respectively. 23,55

4. Angiogenesis

Angiogenesis is important to the development of cancer. The formation of new blood vessels not only supplies the tumor with nutrients and oxygen, but also facilitates metastasis to secondary sites through the hematological route. The growth of all solid tumors is dependent on angiogenesis, in which they are unable to grow larger than a few millimeters in diameter without recruitment of their own vascular bed. A number of miRNAs are found to regulate angiogenesis in colorectal cancer. For instance, miR-107, a miRNA induced by p53 activation, has been shown to suppress tumor angiogenesis, tumor growth, and tumor VEGF expression in mice. 56 miR-145, a miRNA frequently downregulated in colon cancer, also downregulates HIF-1 and VEGF expression to repress angiogenesis by targeting p70S6K1, a downstream signal
mediator of the PI3K/Akt/mTOR cascade. On the contrary, miR-194 inhibits thrombospondin-1 and promotes angiogenesis. These findings indicate that miRNAs are involved in both the positive and negative regulation of angiogenesis.

CLINICAL APPLICATIONS OF miRNA FOR THE MANAGEMENT OF GASTROINTESTINAL CANCERS

1. Disease Susceptibility Markers

Polymorphisms of miRNA genes or miRNA-binding sites have been found to be associated with gastrointestinal cancers or their pre-malignant lesions. In this respect, a polymorphism of miR-27a is associated with an increased risk for gastric mucosal atrophy, a condition predisposing subsequent development of intestinal metaplasia and gastric adenocarcinoma in the presence of H. pylori infection. miRNA-196a-2 gene polymorphism is also associated with increased gastric cancer risk. For prediction of colorectal cancer risk, polymorphisms within the predicted miRNA-binding sites of CD86 and INSR have been implicated.

2. Diagnostic Markers

Detection of pre-neoplastic lesions or early-stage cancers allows curative surgical resection before progression to the advanced inoperable stage. Identification of serum and/or stool miRNA markers has been found useful for early diagnosis of gastrointestinal cancers. The plasma levels of four miRNAs, namely, miR-17-5p, miR-21, miR-106a, miR-106b, are found to be higher in gastric cancer patients. When combined with let-7a, which is found to be lower in gastric cancer patients, the value of the area under the receiver-operating characteristic curve can achieve as high as 0.879 for distinguishing gastric cancer patients and healthy subjects. Another study also confirmed that the combined measurement of miR-17 and miR-106a could achieve the value of the area under the receiver-operating characteristic curve as 0.741 for identifying gastric cancer patients. For colorectal cancer, our group has previously demonstrated that the plasma concentrations of both miR-17-3p and miR-92a were significantly elevated in cancer patients. In this regard, miR-92a could yield a receiver operating characteristic area of 88.5% when a cut-off of 240 relative to RNU6B small nuclear RNA expression was used. Aside from blood, stool is a valuable source for detection of colorectal cancer-derived miRNA since colonocytes are constantly exfoliated from the intestinal tract. To this end, our group has demonstrated that patients with colorectal cancer or polyp had a higher miR-92a level compared with normal controls. Subsequent validation suggests that miR-92a could achieve a sensitivity of 71.6% and 56.1% for identifying colorectal cancer and polyp, respectively, with a specificity of 73.3%. In addition miR-92a, another group has shown that higher fecal levels of miR-21 and miR-106a were found in patients with adenomas and colorectal cancer. These findings indicate that serum and stool miRNAs are useful for screening gastrointestinal cancers.

3. Prognostic Markers

miRNAs have been used to predict the outcome of patients with gastrointestinal cancers. For instance, the expression of seven miRNAs (miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p and miR-126) is associated with the relapse-free and the overall survival of gastric cancer patients. In addition, miR-27a is associated with lymph-node metastasis whereas miR-20b, miR-150, miR-218 and miR-451 predict survival. For colorectal cancer, high expression of miR-21 is associated with lymph node positivity, the development of distant metastases and advanced tumor, node, metastasis staging as well as shorter disease-free interval and poor therapeutic outcome. miR-320 and miR-498 levels are also correlated with recurrence-free survival in stage II colon cancer patients. In colorectal cancer, correlations between miRNA expression or polymorphisms in miRNA genes or miRNA-binding sites and treatment response have also been documented. For example, a polymorphism of let-7-binding site in the KRAS gene is related to the responsiveness to cetuximab in metastatic colorectal cancer patients with wild-type KRAS. The expression levels of let-7g and miR-181b also predict chemoresponse to S-1 (a 5-fluorouracil-based antimetabolite). These findings suggest that miRNAs can be used for prognostication and prediction of treatment response during the management of gastrointestinal cancers.

CONCLUDING REMARKS & FUTURE PERSPECTIVES

In many Asia-Pacific countries, the incidence of colorectal cancer is increasing while the incidence of gastric cancer remains high. Further understanding the pathogenesis of these conditions is required for the development of novel therapeutic agents. Increasing evidence indicates that the dysregulation of miRNA plays an important role in the development of gastrointestinal cancers. In this regard, miRNA regulates a number of cellular processes central to tumorigenesis, including cell proliferation, apoptosis, cellular motility and invasiveness, and angiogenesis. Elucidating the interactions between miRNAs and the cellular signaling networks will shed new light on the complexity of tumorigenesis and help identify novel therapeutic targets. In particular, further characterization of miRNA expression in different subclones within single tumor tissues (i.e., metastatic versus non-metastatic; stem cell-like versus non-stem cell-like, drug-resistant versus drug-sensitive etc.) will be a future direction of miRNA research. Pertinent to clinical practice, accumulating data from miRNA studies has hinted at the potential applications of miRNAs.
as markers for risk stratification, diagnosis, prognostication, and prediction of treatment response in patients with gastrointestinal cancers. The delivery of tumor-suppressing miRNAs or antagonism of oncogenic miRNAs may also open up novel therapeutic avenues. However, the pleiotropic effect of miRNAs may limit their manipulation at the systemic level. Tissue-specific delivery of miRNAs or their antagonists is therefore currently an area of intense investigation. In this regard, intestinal delivery of small RNA by bioengineered probiotic bacteria has been reported.73 The same approach may be adopted for the delivery of miRNAs or their antagonists in the gastrointestinal tract. It is anticipated that, with more understanding, miRNA-based therapeutics will become the latest additions to the armamentarium to fight against cancer in humans in the near future.

REFERENCES

MicroRNA Dysregulations in Gastrointestinal Cancers