

Role of microRNAs in solid tumors

Rie Hamano, Hideshi Ishii, Hiroshi Miyata, Yuichiro Doki, Masaki Mori

Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

Abstract

Accumulating experimental evidence indicates that microRNAs play important roles in various biological processes, such as cell differentiation, proliferation, metabolism and apoptosis. In addition, several reports concluded that altered expression of specific microRNA genes contributes to the initiation and progression of cancer. Here, we summarize the current knowledge about aberrant expression of various microRNAs in human solid cancers (e.g., lung, breast, and gastric cancers), their target proteins, and the relationship between their expression and response to chemotherapies. We also review the potential for using microRNAs as biomarkers for the diagnosis and cancer therapy. The development of treatment strategies against human solid cancers based on the profile and/or certain features of microRNAs is promising.

What is microRNA?

MicroRNAs are noncoding, single-stranded RNAs, 18-25 nucleotides long, and were first reported in *Caenorhabditis elegans* in 1993.¹ Subsequent studies led to the identification of microRNAs in human RNA,² as well as to the understanding of their mechanisms of action. Most human miRNAs are found within introns of either protein-coding or noncoding mRNA transcripts,³ and they do not code for any protein although they are RNA sequences.

MicroRNA genes are generally transcribed by RNA polymerase II in the nucleus to form pri-miRNA transcripts. These are processed into pre-miRNAs by a microprocessor complex, which contains the RNase III enzyme Drosha⁴ and DGCR8.⁵ Exportin5 and RanGTP⁶ transport the pre-miRNAs from the nucleus to the cytoplasm, where they are further processed by the RNAase III enzyme Dicer.⁷ The mature miRNA is retained in RISC (RNA-induced silencing complex)⁸ and it is currently understood that microRNAs mainly bind to the 3' untranslated region (UTR) of their target mRNAs. However, recent studies have reported that microRNAs do not only bind to 3'UTR but

also to 5'UTR^{9,10} or open reading frame (ORF)^{11,12} of the target mRNA. By binding to the 3'UTRs, 5'UTR or ORF of target mRNAs, microRNAs regulate the translation of proteins from mRNA or degrade the mRNA itself.¹³ While microRNAs are thought to repress the translation of target mRNAs, recent results demonstrated that microRNAs can activate the expression of the target genes.¹⁴ In the same study, microRNA was reported to be essential for translation activation under growth arrest conditions. Regulation of translation by microRNAs might change from repression to activation depending on the cell cycle.

In addition, because microRNA can bind even to mRNA that is not partially complementary,¹⁵ microRNA and mRNA do not correspond one-to-one,¹⁶ such that one microRNA may regulate several mRNAs or one mRNA may be regulated by several microRNAs. For example, in human gliomas, miR-34a inhibits the expression of multiple oncogenes (e.g., c-Met, Notch1/Notch-2 and CDK6) by binding to their 3'-UTR and suppressing tumor growth.¹⁷ Thus, these microRNAs potentially regulate approximately 30% of all genes encoding human proteins¹⁸ and appear to achieve a wide range of cell functions, such as cell generation, differentiation, and proliferation.

Aberrant expression of microRNAs in solid cancers

With regard to the relationship between microRNA and cancer, the initial studies reported that B-cell chronic lymphocytic leukemia is associated with downregulation or deletion of miR-15 and miR-16 genes.¹⁹ Other studies subsequently showed that more than half of the microRNAs were located near the unstable DNA region, where chromosomal deletions or amplifications associated with cancer in large the majority of cancer cells.²⁰ Thus, in cancer tissues, detailed profiling of microRNA should be informative and useful for evaluation of the cancer properties. In fact, it is reported that the expression levels of microRNAs vary widely depending on the cancer type and degree of differentiation⁹ and that cancers can be even classified according to the microRNA profile, but not the mRNA profile.²¹

MicroRNAs include both microRNAs that act to inhibit cancer and microRNAs that conversely target tumor suppressor genes and act like oncogenes. To date, numerous reports have examined the aberrant expression of microRNAs and the association between the level of microRNA expression and prognosis in a number of human carcinomas. Table 1 lists the major microRNAs with reported aberrant

Correspondence: Masaki Mori, Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan.
Tel: +81.6.6879.3251 - Fax: +81.6.6879-.3259.
E-mail: mmori@gesurg.med.osaka-u.ac.jp

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expression in solid cancers. To study the relationship between microRNAs and cancer, it is important to examine not only aberrant expressions of microRNAs in carcinomas but also the gene targeted by these microRNAs and to understand their overall roles in cancer. For example, miR-21 is a typical oncogene microRNA whose aberrant expression has been confirmed in various cancers such as breast cancer,²² lung cancer,²³ esophageal cancer,²⁴ colorectal cancer,²⁵ pancreatic cancer,²⁶ and hepatocellular carcinoma.²⁷ Interestingly, the Bcl-2²² and PTEN²⁷ genes are target genes of miR-21, and the oncogene-like function of miR-21 is mediated through the suppression of such tumor suppressor genes.

Lung cancer

One major microRNA, the let-7 family, was first reported to alter the prognosis of patients with lung cancer.²⁸ Oncogenes such as RAS 29) and HMGA2³⁰ are already known as target genes of the let-7 family. In 2008, the first microRNA-knockout mouse was reported, the miR-17-92 knockout mouse, which exhibited hypoplasia of the lungs and B lymphocytes.³¹ MiR-17-92 may also be involved in the process of lung carcinogenesis, and further studies are desirable. In fact, several reports have described the relationship between the expression of miR-17-92 and lung cancer.³²⁻³⁵ On the other hand, the expression of microRNA was recently reported to correlate with smoking.³⁶ Based on the relationship between smoking and lung cancer, further studies are needed to determine the relationship between smoking and microRNA expression. It is anticipated that such studies will allow the design of new approaches for cancer treatment.

Breast cancer

Breast cancer is a major cause of cancer mortality in women,³⁷ and one of the cancers most studied in relation to microRNA. The aberrant expression of many microRNAs has been reported (Table 1). Several studies reported the association between stem cells or cancer stem cells and microRNAs, such as the let-7 family,³⁸ miR-200c,³⁹ and miR-30,⁴⁰ in breast cancer. Furthermore, it is interesting that the number of studies conducted using a murine breast cancer model has been increasing relative to studies on other cancers. One study showed that miR-31 can impede local invasion and suppress metastasis from primary breast tumor *in vivo* and that the expression level of miR-31 correlates inversely with metastasis in human breast cancer.⁴¹ Another study found low expression levels for miR-126 and miR-335 in primary human breast tumors and restoration of the expression of these microRNAs significantly reduced bone metastases *in vivo*.⁴²

Esophageal cancer

Enzymes that contribute to the biogenesis of microRNA in esophageal cancer were first reported in 2006.⁴³ However, there are few reports that have described the relationship between esophageal cancer and aberrant expression of microRNA, compared with other solid tumors (Table 1). This may be due to the difficulty in collecting tissue samples from patients with esophageal cancer because esophagectomy is mostly performed in limited number of institutions. In this regard, a recent study using 70 tissue samples of esophageal cancer collected from several centers in three countries found that up-regulation of miR-21 expression and down-regulation of miR-375 expression correlated significantly with poor prognosis.⁴⁴ Further studies are needed to explore the potential therapeutic effects of microRNAs, such as improvement in sensitivity to radio- and chemo-therapy.

Gastric cancer

The expression of microRNA in gastric cancer was first reported in 2006 in a study that used microarray analysis;⁴⁵ the results showed aberrant expression of 28 microRNAs (22 up-regulated and 6 down-regulated). Gastric cancer includes various histopathological subtypes, such as three degrees of differentiation, mucinous, papillary and signet ring cell, and microRNAs are expressed differentially in this cancer according to histopathological subtype.⁴⁵ Thus, detailed analysis based on classification of histopathological types is necessary for proper analysis of aberrant expression of microRNA in gastric cancer. Although the number of studies on microRNA in gastric cancer is smaller than colorectal cancer and breast

cancer, reports published in 2010 indicate increased interest in the aberrant expression of microRNA in this type of cancer (Table 1).

Colorectal cancer

Similar to breast cancer, the expression of microRNA, including aberrant expression, in colorectal cancer has been the topic of several studies (Table 1). For example, among patients with stage II colorectal cancer, those with high expression of miR-320 and miR-498 are considered to have better relapse-free survival than patients with low expression.⁴⁶ The same report indicated that analysis of the expression of a combination of several microRNAs can predict relapse with 81% accuracy rate, suggesting the potential of microRNA as a biomarker of recurrence. Another feature of colorectal cancer is the association between the expression of microRNAs and the p53 pathway.⁴⁷⁻⁵¹

Hepatocellular carcinoma

Several reports have described the aberrant expression of microRNAs in hepatocellular carcinoma (HCC) (Table 1). The expression of microRNA is also reported to be associated with HBV and HCV infections^{52,53} which are closely related to HCC, and the association with hepatocarcinogenesis has been indicated.⁵⁴ Reduced expression of miR-122 in a chimpanzee model of HCV hepatitis/HCC was reported to result in successful control of HCC,⁵⁵ and the clinical application to humans is greatly anticipated.

Pancreatic cancer

Pancreatic cancer is one of the most malignant cancers, and ranks eighth among the causes of death worldwide.³⁷ In addition to searching for aberrant expression of microRNA in pancreatic cancer (Table 1), analysis of the clinical significance of microRNA on early detection of cancer and the therapeutic outcome would be desirable. In this regard, it has been reported that profile analysis of microRNA expression can differentiate pancreatic cancer from chronic pancreatitis,⁵⁶ which is sometimes difficult to distinguish from pancreatic cancer. In fact, the expression of miR-196a-2 has already been used as a marker for differentiating pancreatic cancer from pancreatitis.⁵⁷ MiR-155 is also reportedly useful for early detection of intraductal papillary mucinous neoplasm (IPMN).⁵⁸

Ovarian cancer

Although there are numerous reports on the aberrant expression of various microRNAs in ovarian cancer (Table 1), interestingly, there are almost no reports on miR-21, which is a typical proto-oncogene. Several studies examined the relationship between microRNA and

sensitivity to cisplatin or paclitaxel chemotherapy, which is often used in clinical settings. For example, among patients with ovarian cancer undergoing cisplatin-based chemotherapy, the complete responders to chemotherapy showed significantly higher expression of let-7i in their tumors compared with the other patients that did not respond completely, and ovarian cancer cells with overexpression of let-7i were more sensitive to cisplatin than those with low expression.⁵⁹

Glioblastoma

Glioblastoma is one of the highest-grade tumor among human intracranial tumors, and aberrant microRNA expression in glioblastoma has been reported in many studies (Table 1). To improve the prognosis of patients with glioblastoma, the development of biomarkers for early detection of glioblastoma, for example circulating microRNAs, is needed. This is particularly important since glioblastoma respond well to treatment with temozolomide, an oral alkylating agent often used for the treatment of intracranial tumors (Table 2).

Anti-cancer therapy and microRNA

In addition to the aforementioned studies that identified aberrant expression of microRNAs in various cancers, it is anticipated that novel anticancer therapeutic strategies will be designed in the future that are based on microRNAs, including chemotherapeutic agents, anti-hormone receptor agents and radiotherapy that target specific microRNAs. Furthermore, changes in the expression levels of microRNAs during any such therapy, relative to the baseline (using microarray analysis), could be also used to predict the sensitivity/resistance of tumors to the anti-tumor agents as well as monitor the response to such treatment.

Table 2-1 shows the relationship between certain microRNAs and the response to chemotherapy. For example, previous studies using microRNA microarray analysis showed down-regulation of 10 microRNAs and up-regulation of two microRNAs in chemoresistant gastric cancer cells compared with parent cells⁶⁰ and down-regulation of two microRNAs and up-regulation of 13 microRNAs in chemoresistant glioblastoma cells compared with parent cells.⁶¹ Another study found significantly low levels of let-7i expression in chemotherapy-resistant patients.⁵⁹ These studies highlight the potential application of microRNAs to the prediction of the tumor response to chemotherapy.

Table 2-2 also lists few microRNAs that were

Table 1. Aberrant expression of microRNA in solid cancers.

| MicroRNA | Target | Expression in tumor | Function | ref |
|---------------------|------------------------|------------------------|---|-----|
| Lung | | | | |
| let-7 | NS | Down | Tumor suppressor | 89 |
| let-7 | HMG2A, K-RAS | Down | Tumor suppressor | 90 |
| let-7 | CDK6, N-RAS | Down | Tumor suppressor | 91 |
| miR-15a,16 | CyclinD1, D2, E1 | Down | cell cycle arrest is induced | 92 |
| miR-17-92 | HIF1 α | NS | miR-17-92 regulates HIF1 α expression under normoxia | 34 |
| miR-17-92 | NS | Up | miR-17-92 is relation to development of B cell and lung | 31 |
| miR-21 | NS | Up | oncogene, EGFR signaling regulates miR-21 expression | 93 |
| miR-21 | NS | Up | miR-21 knock-out mice suppresses Tumor development | 94 |
| miR-29 | DNMT3A, 3B | Down | Tumor suppressor | 95 |
| miR-128b | EGFR | NS | miR-128b LOH is positive prognostic factor | 96 |
| miR-145 | Mucin1 | Down | Tumor suppressor | 97 |
| miR-221, 222 | PTEN, TIMP3 | Up | Oncogene | 98 |
| miR-488, 503, 647 | NS | NS | miR expression pattern to predict recurrence | 99 |
| Breast | | | | |
| let-7 | HRAS, HMG2A | Down | Tumor suppressor | 38 |
| miR-9 | CDH1 | Up | Oncogene | 100 |
| miR-10b | RHOC | Up | Oncogene | 101 |
| miR-10b | HOXD10 | Up | Oncogene | 102 |
| miR-17/20 | IL-8, CK8, CXCL1 | Down | Tumor suppressor | 103 |
| miR-21 | PDCD4 | Up | Oncogene | 104 |
| miR-29a | TTP | Up | Oncogene | 105 |
| miR-30 | Ubc9, ITGB3 | Down | Tumor suppressor | 40 |
| miR-31 | F2d3, ITGA5, MMP6 etc. | Down | Tumor suppressor | 41 |
| miR-126, 335 | SOX4, Tenascin | Down | Tumor suppressor | 42 |
| miR-146a,b | IRAK1, TRAF6 | Down | Tumor suppressor | 106 |
| miR-193b | uPA | Down | Tumor suppressor | 107 |
| miR-200family, 205 | ZEB1, SIP1 | NS | miR-200 family regulate ZEB1 and SIP1 | 108 |
| miR-200c | BMI1 | Down | Tumor suppressor | 39 |
| miR-373, 520c | CD44 | Up | Oncogene | 109 |
| miR-661 | Nectin-1, StarD10 | Up | Oncogene regulated by SNAI1 | 110 |
| Esophagus | | | | |
| miR-10b | KLF4 | Up | Oncogene | 111 |
| miR-16, 30e, 200a | NS | Up | Oncogene | 112 |
| miR-21 | PDCD4 | Up | Oncogene | 24 |
| miR-21, 375 | NS | miR-21: up, -375: Down | miR-21: oncogene, miR-375: Tumor suppressor | 44 |
| miR-106b | p21 | Up | Oncogene | 113 |
| miR-133a,b,145 | FSCN1 | Down | Tumor suppressor | 114 |
| miR-196a | ANXA1 | Up | Oncogene | 115 |
| miR-373 | LATS2 | Up | Oncogene | 116 |
| Stomach | | | | |
| let-7g,miR-214, 433 | NS | miR-422: | let-7, miR-422: Tumor suppressor; miR-214: oncogene | 117 |
| miR-9 | NF- κ B | Down | Tumor suppressor | 118 |
| miR-9, 433 | RAB34, GRB2 | Down | Down-regulated in gastric cancer | 119 |
| miR-23a | IL-6R | Up | Oncogene | 120 |
| miR-31 | NS | Down | Down-regulated in gastric cancer | 121 |
| miR-101 | EZH2, Cox2, Mcl-1, Fos | Down | Tumor suppressor | 122 |
| miR-126 | Crk | Down | Tumor suppressor | 123 |
| miR-129 | CDK6 | Down | Tumor suppressor | 124 |
| miR-129-2 | SOX4 | Down | Tumor suppressor | 125 |
| miR-130b | RUNX3 | Down | Tumor suppressor | 126 |
| miR-141 | NS | Down | Tumor suppressor | 127 |
| miR-181c | NOTCH, KRAS | Down | Tumor suppressor | 128 |
| miR-212 | MeCP2 | Down | Tumor suppressor | 129 |
| miR-218 | Robo1 | Down | Tumor suppressor | 130 |
| miR-218 | ECOP | Down | Tumor suppressor | 131 |
| miR-372 | LATS2 | Up | Oncogene | 132 |
| miR-375 | PDK2, 14-3-3 | Down | Tumor suppressor | 133 |
| miR-421 | CBX7, RBMXL | Up | Up-regulated in gastric cancer | 134 |

Continued next page.

Table 1. Continued from previous page.

| | | | | |
|----------------|----------------------------------|-------------------------------|---|-----|
| Colon | | | | |
| miR-16 | Wip1 | Down | Down-regulated in colon cancer | 47 |
| miR-18* | KRAS | Down | Tumor suppressor | 135 |
| miR-21 | CDC25A | Up | Oncogene | 136 |
| miR-34a | E2F | Down | Tumor suppressor | 137 |
| miR-106a | E2F1 | Down | Tumor suppressor | 138 |
| miR-107 | HIF1 β | Down | Tumor suppressor | 48 |
| miR-143 | DNMT3A | Down | Tumor suppressor | 139 |
| miR-145 | IRS1 | Down | Tumor suppressor | 140 |
| miR-155 | MSH1, MSH2 | Up | Oncogene | 141 |
| miR-192 | NS | NS | Proliferative effect of miR-192 depends on p53 | 50 |
| miR-196a | NS | Up | Oncogene | 142 |
| miR-320, 498 | NS | Down | Tumor suppressor | 46 |
| miR-675 | RB | Up | Oncogene | 143 |
| Liver | | | | |
| miR-18a | ER α | Up | Oncogene | 144 |
| miR-21 | PTEN | Up | Oncogene | 27 |
| miR-26a | NS | Down | Tumor suppressor | 145 |
| miR-101 | Mcl-1 | Down | Tumor suppressor | 68 |
| miR-122 | CyclinG1 | Down | Tumor suppressor | 146 |
| miR-122 | NS | Down | Tumor suppressor | 147 |
| miR-151 | PhoGDIA | Up | Oncogene | 148 |
| miR-181b | TIMP3 | Up | Oncogene | 149 |
| miR-193b | Mcl-1 | NS | HCV proteins alter miR expressions | 53 |
| miR-196 | Bach1 | NS | miR-196 inhibits HCV expression | 54 |
| miR-221 | CDKN1C/p57, CDKN1B/p27 | Up | Oncogene | 150 |
| miR-221 | Bmf | Up | Oncogene | 151 |
| miR-222 | PPP2R2A | Up | Oncogene | 152 |
| miR-223 | STMN1 | Down | Tumor suppressor | 153 |
| Pancreas | | | | |
| miR-21 | NS | Up | Oncogene | 154 |
| miR-27a | Sprouty2 | Up | Oncogene | 155 |
| miR-96 | KRAS | Down | Tumor suppressor | 156 |
| miR-107 | CDK6 | Down | Tumor suppressor | 157 |
| miR-146a | EGFR, IRAK1, NF κ B, MTA2 | Down | Tumor suppressor | 158 |
| miR-155 | TP53INP1 | Up | Oncogene | 159 |
| miR-196a-2 | NS | Up | Oncogene | 57 |
| miR-210 | EFNA3 | Up | Oncogene | 160 |
| Ovary | | | | |
| let-7i | NS | | Tumor suppressor | 59 |
| miR-9, 223 | NS | miR-9: down, miR-223: down | miR-9: Down-regulated, miR-223: up-regulated in recurrent ovarian cancer | 161 |
| miR-15a, 16 | Bmi-1 | Down | Tumor suppressor | 162 |
| miR-20a | APP | Up | Oncogene | 163 |
| miR-27a | NS | Up | Oncogene | 164 |
| miR-31 | CEBPA, STK40, E2F2 | Down | Tumor suppressor | 165 |
| miR-34b, 34c | NS | Down | Tumor suppressor | 166 |
| miR-125a | ARID3B | Up | Oncogene | 167 |
| miR-185 | Six1 | Down | Tumor suppressor | 168 |
| miR-199a | IKK β | Down | Tumor suppressor | 169 |
| miR-199a, 214 | NS | Up and down | Twist1 regulates miRs | 170 |
| miR-200a, 200b | ZEB1,2 | Up | up-regulated in ovarian cancer | 171 |
| miR-210 | E2F3 | NS | miR-210 is a key regulator of hypoxia | 172 |
| miR-221, 222 | CDKN1C | Down | Tumor suppressor | 173 |
| Glioblastoma | | | | |
| miR-7 | EGFR | Down | Tumor suppressor | 174 |
| miR-10b | RhoC, uPAR | Up | Oncogene | 175 |
| miR-17-92 | Smad, etc. | Up | Oncogene | 176 |
| miR-17-92 | CTGF | Up | Oncogene | 177 |
| miR-21 | NS | Up | Oncogene | 178 |
| miR-26a | PTEN, RB1, MEKK2 | Up | Oncogene | 179 |
| miR-34a | NC | Down | Tumor suppressor | 17 |
| miR-128 | Bmi1 | Down | Tumor suppressor | 180 |
| miR-153 | Bcl-2, Mcl-1 | Down | Tumor suppressor | 181 |
| miR-196 | NC | Up | High expression shows poorer survival. | 182 |
| miR-221, 222 | p27, p57 | Down | Tumor suppressor | 183 |
| miR-222, 339 | ICAM1 | Up | MiRs correlate with CTL-mediated cytotoxicity | 184 |
| miR-296 | HGS | Up | miR-296 contributes to angiogenesis | 185 |

NS; not stated

reported to show changes in their expression during cancer treatment. For example, significant reductions in let-7a and let-7b expression levels, relative to the baseline levels, were noted at 8 h after irradiation in lung cancer.⁶² where a significant increase in miR-34 expression was monitored following irradiation-induced DNA damage⁶³ in breast cancer tissue. The development of resistance to chemotherapy is also a problem during cancer treatment. In the cancer stem cell theory, the pluripotent and self-replication properties of the stem cells affect resistance to chemotherapy^{38,64} while microRNAs are known to regulate stem cell functions.⁶⁵⁻⁶⁷ Thus, microRNAs seem to affect

the stability of resistance to antitumor therapies in cancerous tissues. In fact, several recent studies described the correlation between resistance to anticancer drugs and expression of microRNAs known to be involved in stem cell functions (Table 2-2). Furthermore, many of microRNAs are known to enhance sensitivity or reduce the resistance to anti tumor therapy. For example, the hematomas in which miR-101 had been introduced showed higher sensitivity to anticancer agents⁶⁸ and the expression of miR-206 correlated inversely with that of estrogen receptor- α .⁶⁹ Table 2-3 lists some MicroRNAs known to influence the sensitivity to anti-cancer therapy.

Regulation of microRNA

Because microRNA regulate the expression of many mRNAs and microRNAs do not correspond one-to-one to mRNA, a comprehensive analysis is required to understand the regulation of such expression. To gain a better understanding of the overall picture of carcinogenesis, including the function of microRNAs, one should understand the mechanisms involved in the regulation of microRNA expression itself. Previous studies proposed that epigenetic mechanisms and other proteins regu-

Table 2. microRNAs related to sensitivity of anti-cancer therapy.

| MicroRNA | Treatment | Target | Function | Year | Ref |
|---|----------------------|--------------|-----------------------------------|------|-----|
| 2-1. MicroRNAs that are associated with response prediction | | | | | |
| Stomach | | | | | |
| miR15a,16 | ADR, VCR, VP16, CDDP | NS | Increase sensitivity | 2008 | 60 |
| Ovary | | | | | |
| let-7i | CDDP | NS | Increase sensitivity | 2008 | 59 |
| Glioblastoma | | | | | |
| miR-195 | Temozolomide | NS | Increase sensitivity | 2010 | 61 |
| 2-2. MicroRNAs those expressions altered during a therapy | | | | | |
| Lung | | | | | |
| let-7b,g | Radiation | NS | Increase sensitivity | 2007 | 62 |
| Several miRs | Radiation | Int J oncol | 22 miRs expression were changed | 2009 | 186 |
| Breast | | | | | |
| miR-34 | Radiation | NS | Decrease sensitivity | 2009 | 63 |
| Pancreas | | | | | |
| miR-22 | Curcumin | ESR1, SP1 | NS | 2008 | 187 |
| 2-3. MicroRNA that influences the sensitivity to anti-cancer therapy | | | | | |
| Lung | | | | | |
| miR-181a, 630 | CDDP | NS | Increase sensitivity | 2010 | 188 |
| miR-181b | CDDP | Bcl2 | Increase sensitivity | 2010 | 189 |
| Breast | | | | | |
| let-7 | Epi-ADM | H-RAS, HMGA2 | Related to tumor initiating cells | 2007 | 38 |
| Esophagus | | | | | |
| miR-27a | ADR, VCR, 5-FU, CDDP | Bcl2, MRP1 | Decrease sensitivity | 2010 | 190 |
| miR-296 | As above | Bax | Decrease sensitivity | 2010 | 191 |
| Stomach | | | | | |
| miR-221, 222 | Radiation | NS | Decrease sensitivity | 2010 | 192 |
| miR-451 | Radiation | MIF | Increase sensitivity | 2009 | 193 |
| Colon | | | | | |
| miR-140 | 5-FU | HDAC4 | Decrease sensitivity | 2009 | 194 |
| miR-143 | 5-FU | NS | Increase sensitivity | 2009 | 195 |
| miR-215 | MTX, TDX | NS | Decrease sensitivity | 2010 | 196 |
| Liver | | | | | |
| miR-26a | IFN α | NS | Decrease sensitivity | 2009 | 197 |
| miR-199a-3p | ADR | mTOR, c-Met | Increase sensitivity | 2010 | 198 |
| Pancreas | | | | | |
| miR-21 | GEM | NS | Decrease sensitivity | 2010 | 199 |
| miR-21 | 5-FU | NS | Decrease sensitivity | 2010 | 200 |
| miR-21 | GEM | NS | Decrease sensitivity | 2009 | 201 |
| Ovary | | | | | |
| miR-27a | TXL | MDR1 | Decrease sensitivity | 2010 | 202 |
| miR-100 | everolimus | MTOR | Increase sensitivity | 2010 | 203 |
| miR-200c | TXL | TUBB3 | Increase sensitivity | 2009 | 204 |
| Glioblastoma | | | | | |
| miR-21 | Temozolomide | Bax, Bcl-2 | Decrease sensitivity | 2010 | 205 |
| miR-21 | VM-26 | LRRFIP1 | Decrease sensitivity | 2009 | 206 |

CDDP, cisplatin; ADR, doxorubicin; VCR, vincristine; VP16, etoposide; MTX, methotrexate; TDX, thymidylate synthase inhibitor Tomudex; GEM, gemcitabine; TXL, taxol; VM-26, Teniposide; NS, not stated.

late the expression of microRNAs as described below.

Epigenetic mechanisms

Epigenetic modification means aberrant gene expression due to DNA methylation or histone deacetylation. DNA methylation occurs in specific genomic areas called CpG-islands, which are commonly present in the promoter area of the gene.⁷⁰ Methylation of CpG-island is triggered by DNA methyltransferases (DNMTs) and histone modifications are catalyzed by histone deacetylases (HDACs) and histone methyltransferases (HMTs). Tumor genes are globally hypomethylated compared with those of normal tissues,⁷¹ and methylation of CpG islands in the gene promoter area results in inactivation of tumor suppressor genes.⁷⁰ Thus, epigenetic modifications could be involved in carcinogenesis, in addition to other well-defined genetic mechanisms, such as gene mutations and loss of deficiency of heterozygosity.

It was demonstrated recently that certain genes, in particular those with hypermethylated promoters, require Dicer to maintain the epigenetic status.⁷² As mentioned above, Dicer is a key enzyme in microRNA biogenesis. That is a first report that shows the correlation between epigenetic changes of DNA and microRNAs.

Then, Several other studies have reported that epigenetic mechanisms regulate the expression levels of microRNAs. For example, the first report in 2006⁷³ showed that abnormal

methylation correlates with miR-127 expression in several cancer cells. Although miR-127 is not expressed in cancer cells, strong upregulation of this microRNA was noted after treatment with chromatin-modifying drugs (which are also DNA demethylating agents and HDAC inhibitors). Another study showed that the oncoprotein AML1/ETO, an acute myeloid leukemia-associated fusion protein, induced heterochromatic silencing of miR-223 by recruiting DNMTs and HDAC1 activities.⁷⁴ These results point to a complex epigenetic regulation of microRNAs. Table 3-1 lists a group of microRNAs known to be regulated by epigenetic mechanism.

On the other hand, new evidence suggests that microRNAs can control the expression levels of DNMTs and HDACs. For example, microRNA members of the miR-29 family directly target DNMT3A and DNMT3B. Enforced expression of the miR-29 family induced reexpression of methylation-silenced tumor suppressor genes in lung cancer cells, which resulted in inhibition of cancer growth in xenograft models.⁷⁵ Other studies showed that miR-1 directly targeted HDAC-4 in murine myoblasts⁷⁶ while miR449a regulated cell growth by repressing HDAC-1 expression in human prostate cancer cells.⁷⁷ Table 3-2 lists few microRNAs known to control epigenetic mechanisms.

The above studies enhance our understanding of aberrant epigenetic mechanisms in cancers and may prove useful in identifying new targets for cancer therapy.

Regulation by other factors

Among the various families of microRNAs, the let-7 family, which is known to have tumor suppressor function, is under the control of LIN28, which is overexpressed in germ cells by RNA-binding proteins, at the stage of Drosha enzyme processing.⁷⁸ The latter study indicated the specificity of the regulatory mechanism of LIN28 to the let-7 family by demonstrating the lack of any inhibitory effects on other microRNA. Dicer, another enzyme involved in the processing of microRNAs, also inhibits the let-7 family and forms a negative feedback loop with let-7 family.⁷⁹ Other studies reported the regulation of microRNAs by other transcription factors, such as p53⁸⁰ and c-myc,⁸¹ suggesting that many factors are intricately involved in the mechanisms that regulate microRNAs in cancers. The number of microRNA-related regulatory factors reported to date is not very large, but it is expected to expand exponentially in the future.

MicroRNAs as biomarkers for cancer

Although many aspects of microRNA formation in the cell remain unclear, it is becoming evident that microRNAs are more stable in the cells than mRNA. Accordingly, it is anticipated that microRNAs may serve as biomarkers of cancer better than mRNA. Historically, intrinsic microRNA levels in the circulation were

Table 3. microRNAs that are regulated by epigenetic gene silencing.

| MicroRNA | Cancer type | Target | Detail | Year | Ref |
|--|--------------------------|-------------------|---|------|-----|
| 3-1. Some microRNAs of which expression controlled by epigenetic mechanism | | | | | |
| let-7a-3 | Ovary | NS | let-7a-3 methylation is associated with survival | 2007 | 207 |
| miR-1 | Liver | FoxP1, MET, HDAC4 | Overexpression in cells treated with 5- AZA | 2008 | 208 |
| miR-9-1 | Breast | NS | Overexpression in cells treated with 5-AZA | 2008 | 209 |
| miR-9, 34b/c, 148a | Various types | oncogenes | Overexpression in cells treated with 5-AZA | 2008 | 210 |
| miR-9, 129, 137 | Colon | NS | Overexpression in cells treated with 5- AZA | 2009 | 211 |
| miR-34b, -34c | Colon | BTG4 | miR-34b/c methylation is frequently observed in cancer cells | 2008 | 212 |
| miR-124a | Colon | CDK6 | Overexpression in cells treated with 5-AZA | 2007 | 213 |
| miR-127 | Bladder | BCL6 | Overexpression in cells treated with 5-AZA | 2006 | 73 |
| miR-129-2 | Ovary | SOX2 | Overexpression in cells treated with epigenetic drugs | 2009 | 214 |
| miR-137a | Colon | LSD1 | miR-137 methylation is specific for cancer | 2010 | 215 |
| miR-223 | Leukemia | NS | AML1/ETO induced heterochromatic silencing of miR-223 | 2007 | 74 |
| miR-370 | Biliary duct | MAP3K8 | Overexpression in cells treated with 5-AZA | 2008 | 216 |
| miR-512-5p | Stomach | Mcl-1 | Overexpression in cells treated with 5- AZA | 2009 | 217 |
| 3-2. Some microRNAs that controls epigenetic mechanism | | | | | |
| miR-1 | Myoblast (not malignant) | HDAC-4 | MiR-1 represses HDAC-4 | 2006 | 76 |
| miR-29 family | Lung | DNMT3a, 3b | Enforced expression restores normal patterns of DNA methylation | 2007 | 75 |
| miR-29b | Leukemia | DNMT3a, 3b | Enforced expression restores normal patterns of DNA methylation | 2009 | 218 |
| miR-148a, b | Various types | DNMT3b | MiR-148 represses DNMT3b | 2008 | 219 |
| MiR-449 | Prostate | HDAC-1 | MiR-449 directly targets HDAC-1 | 2009 | 77 |

5-AZA, 5-Aza-20-deoxycytidine; NS, not stated.

found to be relatively stable against endogenous RNAase.⁸² Subsequent studies reported higher blood miR-195 and let-7 expression levels in patients with breast cancer compared with healthy subjects and that these expression levels fell after surgical excision of the tumor.⁸³ Furthermore, the expression levels of miR-29a and miR-92a were also found to increase with the stage of colorectal cancer,⁸⁴ suggesting their potential suitability as a cancer screening tool.

Recent studies have reported measurement of microRNAs in other body fluids in addition to blood, such as feces⁸⁵ and sputum.⁸⁶ For example, significantly higher expression levels of miR-21 were found in the sputum of patients with lung cancer compared with healthy subjects, indicating high sensitivity and specificity.⁸⁷ On the other hand, the expression levels of miR-125a and miR-200a in the saliva were significantly lower in patients with oral cancer than healthy subjects.⁸⁸ Further studies are needed to design simple and noninvasive assays that accurately measure microRNAs collected from human tissues. Such methods will be helpful for screening of cancer or assessment of the therapeutic effects of anti-cancer treatment.

Future perspective of microRNA

As noted earlier, microRNA are expected to play a major role in the future as biomarkers for screening cancer, predicting response to therapies, and assessing the effect of treatment.

Progress is also anticipated in the development of new microRNA-based anti-cancer therapies. Such therapies could be designed to restrict cancer growth by applying the mRNA regulatory function of microRNA to inhibit oncogenes or activate tumor suppressor genes. Alternatively, new therapies could be designed based on the finding of increased potency of standard chemotherapies when combined with microRNAs.

We are only just beginning to understand microRNAs and their hidden potential. Worldwide research on microRNAs, including clinical application, is currently underway. Treatment strategies against solid cancers based on profile or features of microRNAs are expected to be developed in the near future.

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