REVIEW

Non-coding RNAs for Medical Practice in Oncology

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Alterations in microRNA (miRNA) and other short or long non-coding RNA (ncRNA) are involved in the initiation, progression, and metastasis of human cancer. The main molecular alterations result from variations in gene expression, which are usually minor but have consequences for a vast number of target protein-coding genes. The causes of the widespread differential expression of ncRNAs in malignant cells compared with normal cells can be explained by the location of these genes in genomic regions associated with cancer, by epigenetic mechanisms, and by alterations in the processing machinery. Expression profiling of human tumors based on the expression of miRNAs and other short or long ncRNAs has identified signatures associated with diagnosis, staging, progression, prognosis, and response to treatment. In addition, profiling has been exploited to identify ncRNAs that may represent downstream targets of activated oncogenic pathways or that target protein-coding genes involved in cancer. Recent studies found that miRNAs and non-coding ultraconserved genes are the main candidates for the elusive class of cancer-predisposing genes and that other types of ncRNAs participate in the genetic puzzle that gives rise to the malignant phenotype. These discoveries could be exploited for the development of useful markers for diagnosis and prognosis in cancer, as well as for the development of new RNA-based cancer therapies. (Keio J Med 60 (4) : 106–113, December 2011)

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Introduction

Non-coding RNAs (ncRNAs) are RNAs that do not encode proteins. Until recently the central dogma of genetics was that RNA played the role of messenger between the gene and the final proteins codified by the gene, and ncRNAs were ignored in the field of genome sequencing.[1,2] In the past decade, genome-wide transcriptional analyses have estimated that most transcribed mammalian genomic sequences are ncRNAs.[3–5] In addition, new ncRNAs have been recognized as important participants in several mechanisms of gene regulation.[5]

These new ncRNAs can be categorized as either short or long. Long ncRNAs (lncRNAs) are processed in full from their templates and are typically >200 nt long; small ncRNAs, conversely, are processed from longer precursors and include microRNAs (miRNAs), small interfering RNAs, piwi-interacting RNAs, tiny RNAs, and cryptic unstable transcripts.[5]

Many classes of ncRNAs have been described in the literature, and their roles in cellular biology are recognized as being sophisticated. miRNAs are well-known ncRNAs molecules that regulate various gene expressions at the RNA level. In addition, lncRNAs such as ultraconserved genes (UCGs) and HOX antisense intergenic RNA (HOTAIR) have been recently characterized.[6,7]

In various human diseases, the alteration of several ncRNA types has been reported. A role for ncRNA in hu-

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man carcinogenesis is most certain, providing evidence for targeting these molecules as anticancer agents. However, at present, a clear relationship with cancer has been reported for only a few ncRNA classes (miRNA, UCG, and HOTAIR). Therefore, the purpose of this review is to describe recent findings on UCGs, HOTAIR, and especially miRNAs and their effect on cancer.

HOTAIR and Ultraconserved Regions (UCRs)

lncRNAs range in size from approximately 200 nt to over 100 kb. Like messenger RNAs (mRNAs), lncRNAs seem to be transcribed mostly by RNA polymerase II, but many lncRNAs do not undergo the subsequent standard mRNA processing steps.[8] Because of this characteristic, lncRNAs often have regulatory functions involving nuclear retention close to transcription sites.[9] Although the mechanisms and functions of lncRNAs are mostly unclear, they seem to mirror those of protein-coding genes.[6]

The mechanism of lncRNA function is generally unknown, but evidence suggests that it is similar to that of HOTAIR, which is itself an lncRNA. HOTAIR regulates the chromatin methylation state of the HOXD locus in *trans* through polycomb repressive complex 2. HOTAIR is related to polycomb repressive complex 2 and lysine-specific demethylase 1/RE1-silencing transcription factor co-repressor 1/RE1-silencing transcription factor protein complexes; HOTAIR coordinates the chromatin targeting of these proteins and it couples histone H3K27 methylation with H3K4 demethylation to create epigenetic gene silencing.[10,11] HOTAIR was recently reported to play a role in cancer metastasis and may be an indicator of poor prognosis in patients with primary breast cancer.[7]

UCGs, one of the new ncRNAs, are transcribed from UCRs[12] and have a tissue-specific expression pattern.[12,13] Researchers became aware of UCRs when the alignments of human, rat, and mouse genomes demonstrated that despite 300 million years of negative selection, some genomic regions remained highly conserved (100% identity).[5,14] In fact, UCRs are even more conserved than coding genes are, and UCRs are now believed to have fundamental functions in vertebrate evolution, including that of mammals.[2,14–16] UCRs are frequently found in fragile genomic regions that are usually associated with cancer, and UCG expression is aberrant in several human carcinomas and leukemias compared to that of their normal tissue counterparts.[12] Deregulated UCG signatures are cancer specific and have prognostic implications. As is also the case for miRNAs, UCGs can act as oncogenes or tumor-suppressor genes, and UCG expression is controlled by miRNAs.[12]

miRNAs

miRNAs are ncRNAs of 19–25 nt in length and are classic examples of ncRNAs. By degrading or blocking translation of mRNA targets, these miRNAs regulate more than 30% of the mammalian genome.[17] miRNAs are also involved in the initiation, progression, and metastasis of human cancer[18,19] and can behave as either oncogenes or tumor suppressors.[17]

miRNA profiling, which has been achieved by various methods, has identified signatures associated with diagnosis, staging, progression, prognosis, and response to treatment of human tumors. Therefore, miRNA profiling represents a new addition to the diagnostic and prognostic tools available for use by medical oncologists. The main paradigms for miRNA involvement in human cancers are summarized in the sections that follow.

Alteration of miRNAs in All Human Malignancies

Alterations of miRNAs, first identified in the most common form of adult leukemia, i.e., B cell chronic lymphocytic leukemia (CLL),[20] have since been detected in many types of human tumors (**Table 1**)[18,20–68]. The main mechanism of alterations of microRNoma (defined as the full complement of miRNAs present in a genome) in cancer cells seems to be aberrant gene expression, which is characterized by abnormal levels of expression of mature and/or precursor miRNA sequences. The causes of the widespread differential expression of miRNA genes between malignant and normal cells can be explained by the genomic location of these genes in cancer-associated regions and genomic alterations,[18] by epigenetic mechanisms, and by alterations in the processing machinery

Several developments in high-throughput miRNA profiling include oligonucleotide miRNA microarrays, as described previously[69]; the use of a bead-based flow cytometric technique[36–38,70]; the quantitative reverse transcription-polymerase chain reaction for precursor miRNA[71] or active miRNA[72,73]; the miRNA serial analysis of gene expression (miRAGE), which combines direct miRNA cloning with serial analysis of gene expression (SAGE)[35–38,73,74]; and the high-throughput array-based Klenow enzyme (RAKE) assay.[75] miRNAs differentially expressed between tumors and their normal tissue counterparts have been identified for several tumor types, including lymphoma,[57] breast cancer,[25] lung cancer,[24] thyroid papillary carcinoma,[76] glioblastoma,[63] hepatocellular carcinoma,[77] colorectal carcinoma,[74] pancreatic tumors,[78] and pituitary adenomas.[79]

miRNA Alterations May Result in Cancer Predisposition

Despite extensive research, the molecular basis for most familial cancers is unknown. Because the extent of miRNA involvement in human cancers is much greater than initially believed, it is tempting to propose that germline mutations or polymorphisms in miRNA genes or interacting sequences from target mRNA might repre-

Table 1 miRNA expression profiles in common types of cancer

miRNA	Target genes	Property	Expression in cancer	References
miR-15a miR-16–1 cluster	Bcl-2, MCL1, Wt-1	TS	Downregulated in CLL	20–23
let-7 family	KRAS, NRAS, c-myc, HMGA2	TS	Downregulated in lung and breast cancers	24–29
miR-29 family	TCL-1, MCL1, CDK6, DNMT3s	TS	Downregulated in CLL, AML, lung cancer, breast cancer, and cholangiocarcinoma	24,25,30–34
miR-34a/b/c	CDK4, CDK6, Bcl-2, cyclinE2, E2F3, c-Met, c-Myc	TS	Downregulated in pancreatic, colon, and breast cancers	35–38
miR-200/141 family	ZEB1, ZEB2	TS	Downregulated in breast, renal clear cell, gastric, and bladder cancers	39–43
miR-155	c-maf, SHIP1	OG	Upregulated in high-risk CLL, AML, lymphomas, colon cancer, lung cancer, and breast cancer	24,25,31,44–51
miR-17–92 family	E2F1, Bim, PTEN	OG	Upregulated in lymphomas and breast, lung, colon, stomach, and pancreatic cancers	51–62
miR-21	PTEN, PDCD4, TPM1	OG	Upregulated in breast, colon, pancreatic, lung, prostate, liver, and stomach cancers; AML; CLL; and glioblastomas	18,30,31,47,51,63–67
miR-372/ miR-373	LATS2	OG	Upregulated in testicular cancer	68

CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; TS, tumor suppressor; OG, oncogene.

sent a mechanism of cancer predisposition (for a review, see Calin et al. 2006[18]). In fact, a germline mutation in the *pri-miR-16–1/15a* precursor in a patient with familial CLL and breast cancer in first-degree family members suggests a possible predisposing effect,[30] but the precise roles of mutations in miRNAs are still unknown. Tumor-specific pri-miRNA sequence abnormalities seem to be a more widespread phenomenon in tumorigenesis, as mutations near the clusters *miR-17–92* on chromosome 13 and *miR-106–92* on chromosome X were described in a mouse model.

These clusters are amplified in human lymphomas[80] and accelerate c-MYC-induced tumorigenesis in a mouse model of B-cell lymphoma,[57] suggesting a possible pathogenetic role for such mutations. Furthermore, germline single nucleotide polymorphisms were identified in two recognition sites in the c-KIT oncogene for *miR-221*, *miR-222*, and *miR-146*, which are all strongly overexpressed in thyroid cancers.[76] Therefore, because the thermodynamics of RNA–RNA binding plays an essential role in a miRNA interaction with its target mRNA, sequence variations influencing this interaction may result in cancer predisposition.

miRNAs as Oncogenes and Tumor Suppressors

The classical models of tumorigenesis postulate alterations in protein coding oncogenes and tumor suppressor genes. miRNAs are also contributors to oncogenesis, functioning as tumor suppressors (as in the case of *miR-15a* and *miR-16–1*[23] and the *let-7* family[26]) or as oncogenes (as in the case of *miR-155*,[51] the *miR17-92* cluster,[57,76] and *miR-21*[51,65]). Relatively minor variations in the levels of expression of a miRNA or mutations that moderately affect the conformation of the miRNA::mRNA pairing could have important consequences for the cell because of the large number of targets of each miRNA. For example, downregulation of the suppressor *miR-15a/miR-16–1* induces overexpression of the B cell lymphoma 2 (BCL2) gene and possibly other genes that may be important for tumorigenesis,[23] while overexpression of oncogenic *miR-17–92* cooperates with *c-MYC* in stimulating lymphomagenesis.[57,76] miRNA alterations in somatic cells can initiate or contribute to tumorigenesis, and miRNA alterations in germline cells may predispose a person to cancer. A paradigm for this model is human B-cell CLL, in which *miR-15a* and *miR-16–1* are located in the most frequently deleted genomic region, are downregulated in the majority of cases, harbor mutations in familial cases, and induce apoptosis in a leukemia mouse model by targeting the antiapoptotic *BCL2* gene.

miRNA Profiling Can Improve Diagnosis of Cancer in Patients

To date, numerous profiling studies using tumors of various histotypes have been published.[51,70,81] Lu et al[70]., using a bead-based flow-cytometric profiling technology performed on a set of 334 samples that included multiple human cancers (mainly leukemias), found that miRNA expression profiles classify human cancers and reflect the developmental lineage and differentiation state of the tumors. Of important diagnostic consequence is that the miRNA-based classifier is better at establishing the correct diagnosis of poorly differentiated samples with non-diagnostic histological appearance than is the protein-coding gene's mRNA classifier. This type of classification represents an important advance in the diag-

nosis of metastatic cancer of unknown primary site, as these patients present with metastases (late-stage disease) without an established primary tumor (i.e., a site where a therapeutically curative or palliative intervention can be performed).

Volinia et al.[51] described a large-scale, detailed microarray analysis using 540 samples from six types of the most frequent solid human cancers and found a common signature composed of 61 miRNAs, mainly overexpressed with respect to corresponding normal tissues. Evidence has been found that the predicted targets for the differentially expressed miRNAs are significantly enriched for protein-coding tumor suppressors and oncogenes, supporting the roles of these genes in tumorigenesis. Of the 228 miRNA genes analyzed, 36 were overexpressed and 21 were downregulated in cancer cells compared with normal cells. Hierarchical clustering analysis showed that this miRNA signature enabled the tumor samples to be grouped on the basis of their tissue of origin, which can greatly help in diagnosis.

miRNA Profiling Can Predict Outcome for Cancer Patients

In most CLL patients, the prognosis is relatively good and, after diagnosis, treatment is started only if poor prognostic markers are evident. Among these markers, those most commonly used are high expression levels of the zeta-chain-associated protein of 70 kDa (*ZAP-70*) gene and the absence of mutations in the immunoglobulin variable-region heavy-chain (*IgVH*) gene. By performing a miRNA profiling screening of 144 CLL patients, researchers found that a unique signature of 13 miRNAs (of the 190 analyzed) differentiated cases on the basis of a good or poor prognosis and on the presence or absence of disease progression.[30] Among the 13 miRNAs, *miR-16–1* and *miR-15a* were expressed at lower levels in patients with a good prognosis, which was in agreement with early reports that 13q14.3 genomic deletions at the locus harboring these genes are favorable prognostic features.

Identification of new prognostic markers could represent a significant advance for the identification of patients who would benefit from more aggressive therapy for lung cancer, which is the leading cause of cancer death in men worldwide. In univariate analyses, expressions of both *miR-155* and *let-7*a-2 were shown to correlate with poor survival in 104 American patients with lung cancer; in multivariate analyses, only the expression of *miR-155* was correlated with poor prognosis when all clinical variables were considered together.[24] In an independent study of 143 Japanese patients with lung cancer, underexpression of *let-7* was found to significantly correlate with a shorter survival time after potentially curative surgery; multivariate analysis showed that underexpression of *let-7* was an independent prognostic factor for the stage of the disease and correlated with significantly shorter survival time.[82]

miRNAs as Therapeutic Targets

Because miRNAs regulate the expression of multiple genes in a disease pathway, miRNAs and the genes they influence can be therapeutic targets. The use of miRNAs as drug therapies is still at the preclinical stage, and the results of clinical and toxicological studies have not yet been published. miRNA mimics were shown to induce cell death and reduce tumorigenic potential of the miR-15a/16 cluster in a leukemia model[21] and of the members of the miR-29 family in a lung cancer model.[33]

Several animal model studies have been reported.[83–86] Systemic administration of anti-miRNA (*miR-122)* oligonucleotide therapeutics in mice and African green monkeys was shown to reduce total plasma cholesterol and hepatitis C viral load. As a result, systemic administration of *miR-122* could reduce liver damage.[83–85] In addition, when the mimic for miR-26a was administered systemically in a murine model of liver cancer, tumor size was clearly reduced.[86] Thus, in addition to confirming the safety and efficacy of anti-miRNA oligonucleotides in a preclinical trial, these studies have established a baseline for their testing in future clinical trials.

Conclusions

There is no question that miRNAs and other ncRNAs are involved in the regulation of tumorigenic pathways involved in tumor development and progression. Whether miRNAs represent the "dark side" of cancer predisposition will be soon be clearly answered by our future studies in a large series of familial cancer patients. miRNAs and other ncRNAs have only recently been identified as new diagnostic and prognostic tools for cancer patients, and the use of miRNAs in cancer therapy represents a potential treatment option for medical oncologists in the near future. The rationale for using miRNAs and other ncRNAs in cancer treatment is that they are natural antisense interactors that regulate many genes involved in eukaryotic survival and proliferation. These discoveries could be exploited for the development of useful markers for diagnosis and prognosis in cancer, as well as for the development of new RNA-based cancer therapies.

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