MicroRNA-Cancer Connection: The Beginning of a New Tale

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Abstract

Cancer initiation and progression can involve microRNAs (miRNA), which are small noncoding RNAs that can regulate gene expression. Their expression profiles can be used for the classification, diagnosis, and prognosis of human malignancies. Loss or amplification of miRNA genes has been reported in a variety of cancers, and altered patterns of miRNA expression may affect cell cycle and survival programs. Germ-line and somatic mutations in miRNAs or polymorphisms in the miRNAs targeted by miRNAs may also contribute to cancer predisposition and progression. We propose that alterations in miRNA genes play a critical role in the pathophysiology of many, perhaps all, human cancers. (Cancer Res 2006; 66(15): 7390-4)

Cancer-Specific MicroRNA Fingerprints

Cancer is a very complex genetic disease characterized by alterations in genes encoding oncogenic and tumor-suppressor proteins [protein coding genes (PCG)]. First described in C. elegans more than a decade ago (1), >3,000 members of a new class of small noncoding RNAs, named microRNAs (miRNAs; ref. 2), have been identified in the last 5 years in vertebrates, flies, worms, and plants, and even in viruses. Functionally, it was shown that miRNAs reduce the levels of many of their target transcripts as well as the amount of protein encoded by these transcripts (3). For several miRNAs, the participation in essential biological processes has been proved, such as cell proliferation control (miR-125b and let-7), hematopoietic B-cell lineage fate (miR-181), B-cell survival (miR-15a and miR-16-1), brain patterning (miR-430), pancreatic cell insulin secretion (miR-373), and adipocyte development (miR-143; for reviews, see ref. 4).

After the identification of two clustered miRNAs as the targets of homozygous and heterozygous deletions and translocations at 13q14.3 in human B-cell chronic lymphocytic leukemias (B-CLL; ref. 5), the question to be answered was how general is the involvement of miRNAs in human cancers. The development of miRNA microarrays was a necessary step for the high-throughput miRNA fingerprint investigation in normal and cancer cells (6). Other technologies, including macroarrays (7), bead-based flow cytometric miRNA expression (8), and quantitative reverse transcription-PCR (9), are now available. What we learned from such expression studies is reshaping the landscape of cancer genomics (Table 1 and included references).

Cancer-specific miRNA fingerprints were identified in every type of analyzed cancer, including B-CLL (10), breast carcinoma (11), primary glioblastoma (12), hepatocellular carcinoma (13), papillary thyroid carcinoma (14), lung cancer (15–17), gastric carcinoma, colon carcinoma (18), and endocrine pancreatic tumors (17). Not only the spectrum of miRNAs expressed in malignant cells is significantly different from that of normal counterpart cells but also miRNA expression profiles better classify poorly differentiated tumors as compared with the mRNA (EST)-based classifier (8). Commonly deregulated miRNAs in different types of solid cancers predict their involvement in fundamental pathways and their interaction with important cancer-specific PCGs (17). Furthermore, such abnormal expression was found not only in malignant cells but also in premalignant stages, such as colon adenomas where miR-143 and miR-145 expression is reduced (18) or in pituitary adenomas, a type of benign tumors displaying deletions at 13q14.3 and reduced expression of miR-16-1 and miR-15a (19). The finding that essentially all indolent CLLs have lost miR-15a/miR-16-1 expression suggests that this event is the initiating event in the pathogenesis of the indolent form of CLL (5, 10, 20). Furthermore, it was shown that miR-221, highly overexpressed in papillary thyroid tumors, is also overexpressed in normal thyroid tissue adjacent to tumors but not in normal thyroid tissues from individuals without clinical thyroid disease (14). Therefore, it seems likely that, in some cases, the cancer-specific miRNA fingerprints represent events involved in the initiation of the malignant process.

What are the causes of the widespread miRNA misexpression in cancers? Although not clearly understood, the origins of such abnormalities seem to be multiple. Many miRNAs reside in genomic regions involved in cancer, including minimal regions of loss of heterozygosity (LOH), minimal amplicons, or breakpoint cluster regions (21). As shown in Table 1, the overexpressed oncogenic miRNAs are located in amplified regions and the downregulated suppressor miRNAs in deleted regions in cancers. The proof that chromosomal rearrangements are causal includes the early report of a masked t(8;17) translocation that resulted in an aggressive B-cell leukemia by overexpressing c-myc oncogene by an unknown mechanism at the moment of identification (22). It was shown later that miR-142 is located at the chromosome 17 breakpoint and that c-Myc was rearranged under the control of the promoter of miR-142 with consequent overexpression. In a precursor B-cell acute lymphoblastic leukemia, an insertion of miR-125b-1 into a rearranged immunoglobulin heavy chain locus was described, suggesting an early involvement in leukemogenesis (23).

Mutations in MiRNAs: A Way to Predispose to Cancer?

In spite of decades of research, the molecular basis for the major fraction of familial cancers is unknown. CLL represents one of the main examples in this regard: a significant portion (10-20%) of patients have a family history of CLL or other hematologic or solid cancers whereas no clear culprit could be found by scanning PCGs (20, 24). Screening the human miRNome for sequence abnormalities located either in the pre-miRNA or in pri-miRNA, a higher frequency of germ-line or somatic mutations (about 15%), as expected by the small size of miRNA genes was found (25).
Furthermore, a germ-line mutation in the pri-miR-16-1/15a precursor in a patient with familial CLL and breast cancer in first-degree members of family suggests a possible predisposing effect. The roles of mutations in miRNAs have still to be elucidated, and tumor-specific pri-miRNA sequence abnormalities seem to be a more widespread phenomenon in tumorigenesis since mutations near the clusters miR-17-92 on chromosome 13 and miR-106-92 on chromosome X were described in a mouse model (26). It was

<table>
<thead>
<tr>
<th>MiRNA</th>
<th>Location</th>
<th>Putative function</th>
<th>CAGR location*</th>
<th>Cancer abnormalities/description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-16-1-15a cluster</td>
<td>13q14.3, intron 4 of DLEU2</td>
<td>Suppress miRNAs</td>
<td>LOH in CLL and prostate cancer</td>
<td>Deleted and down-regulated in the majority of B-CLLs</td>
<td>(5)</td>
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<td></td>
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<td>Reduced expression in the majority of DLBCLs</td>
<td>(31)</td>
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<td>Down-regulation in pituitary adenomas</td>
<td>(19)</td>
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<td>Reduced expression associated with good prognosis in B-CLL</td>
<td>(25)</td>
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<td>Germ-line mutations in the primary transcript</td>
<td>(25)</td>
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<td>miR-16-1/15a in B-CLLs</td>
<td>(25)</td>
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<td>Exogenous restoration in leukemia cells of miR-16/15 induces apoptosis by directly targeting BCL2</td>
<td>(36)</td>
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<tr>
<td>miR-145</td>
<td>5q32, intergenic</td>
<td>Suppress miRNA</td>
<td>LOH in MDS (5q– syndrome)</td>
<td>Reduced accumulation in colon adenomas and carcinomas</td>
<td>(18)</td>
</tr>
<tr>
<td>let-7 family</td>
<td>various</td>
<td>Suppress miRNAs</td>
<td>LOH in lung cancers</td>
<td>Reduced expression in breast cancers</td>
<td>(11)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Reduced expression associated with shortened postoperative survival</td>
<td>(15)</td>
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<td>let-7a-1 expression correlates with poor survival of lung cancer patients</td>
<td>(16)</td>
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<td>let-7 regulates RAS oncogene expression in lung tumors</td>
<td>(33)</td>
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<td>miR-155</td>
<td>21q21.3, exon 3 of noncoding RNA BIC</td>
<td>Oncogenic miRNA</td>
<td>not reported</td>
<td>High expression of precursor miR-155/BIC in pediatric BL, but lack of BIC and miR-155 expression in adult BL.</td>
<td>(38, 41)</td>
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<td>miR-155 overexpressed in B-cell lymphomas, significantly higher levels in DLBCL with poor prognosis (activated B-cell phenotype)</td>
<td>(31, 39)</td>
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<td>Increased expression of miR-155 in Epstein-Barr transformed lymphoblastoid cell lines</td>
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<td>High expression of both BIC and miR-155 in Hodgkin, primary mediastinal and DLBCL lymphomas</td>
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<td>Overexpression in breast cancers</td>
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<td></td>
<td></td>
<td>miR-155 overexpression correlates with poor survival in lung cancers</td>
<td>(16)</td>
</tr>
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<td>miR-17-92 cluster</td>
<td>13q31.3, intron 3 C13orf25</td>
<td>Oncogenic miRNA</td>
<td>AMPLIF in follicular lymphomas</td>
<td>Target of genomic amplification in malignant lymphomas</td>
<td>(27, 28)</td>
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<td>Overexpressed in lung cancers; the miRNA cluster, but not the host C13orf25 gene, enhances cell proliferation.</td>
<td>(40)</td>
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<td>Primary transcripts overexpressed in lymphomas, but not in colorectal carcinomas; enforced expression acted with the c-Myc expression to accelerate tumor development in mouse B-cell lymphomas.</td>
<td>(29)</td>
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<td>miR-21</td>
<td>7q23.2, 3’UTR VMP1</td>
<td>Oncogenic miRNA</td>
<td>AMPLIF in neuroblastoma and breast cancer</td>
<td>Negative regulatory feed-back loop c-Myc/ miR-17-5p-miR-20a/E2F1</td>
<td>(37)</td>
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<td>Elevated levels in glioblastoma primary tumors and cell lines; increased apoptotic cell death after miR-21 knockdown in glioblastoma cells</td>
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<td></td>
<td></td>
<td></td>
<td>Overexpression in breast cancers</td>
<td>(11)</td>
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</tbody>
</table>

Abbreviations: B-CLL, B-cell chronic lymphocytic leukemia; BIC, noncoding RNA gene; BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; DLEU2, noncoding RNA gene; VMP1, vacuole membrane protein 1.

*CAGR, cancer-associated genomic regions (as in ref. 21).
shown that the cluster miR-17-92 is amplified in human lymphomas (27, 28) and accelerates c-Myc-induced tumorigenesis in a mouse model of B-cell lymphoma (29), suggesting a pathogenic role of such mutations.

As the thermodynamics of RNA-RNA binding plays essential roles in the miRNA interaction with the target mRNA, it is supposed that sequence variations influencing this interaction will be identified in cancers. Thyroid cancers in which the up-regulation of miR-221, miR-222, and miR-146 was the strongest showed dramatic loss of KIT oncogene and, in half of the cases, the down-regulation was associated with germ-line single-nucleotide polymorphisms in the two recognition sites in KIT for these three miRNAs (14). It has to be noted that thyroid papillary carcinoma is a type of cancer with high familiarity without known genetic bases. As the 3' untranslated region (UTR) of PCGs was scarcely screened for mutations/polymorphisms, it is possible that the extent of such abnormalities might be much larger than initially thought. Further strengthening possible roles of polymorphisms in altering the function of miRNAs, a study in Japanese normal subjects screened for single-nucleotide polymorphisms in the genomic regions corresponding to 173 precursor miRNAs found a polymorphism in the mature miR-30c-2 sequence that may alter target selection and exert biological effects (30). Making the story more intriguing, this miRNA is a member of a common expression signature characterizing several solid cancers (17). Putting all these data together, it is tempting to propose that germ-line mutations or polymorphisms in miRNA genes or interacting sequences in target mRNA might represent a newly described mechanism of cancer predisposition. Further identification of sequence or expression variations in miRNAs in a large series of familial cancer patients is needed to clearly prove this hypothesis.

**MiRNAs: From the Scientist Bench to the Patient Bedside**

It is well known that PCGs with important cancer connections are used also as diagnosis markers and therapy targets. If the miRNAs are active players in human oncogenesis, then they will have an effect on the diagnosis and prognosis of cancer (Table 1). In fact, evidence that miRNAs represent new diagnostic and prognostic factors in human cancers is rapidly accumulating. In B-CLL, a unique miRNA signature is associated with prognostic factors and with the time from diagnosis to initiation of therapy (25). In diffuse large B-cell lymphoma, independent studies revealed that significantly higher levels of miR-155 occur in cases with poorer prognosis (an activated B-cell phenotype) than in those with the germinal center phenotype (31, 32). Expression of members of let-7 family correlates with postoperative survival in lung cancer, the group of patients with reduced expression showing significantly shorter survival after potentially curative resection of the tumor (15). In lung adenocarcinomas, high miR-155 and low let-7a expression correlates with poor survival (16). In breast carcinomas, miRNA expression was correlated with specific biopathologic features, such as estrogen and progesterone receptor expression (the members of miR-30 family), or tumor stage (miR-213 and miR-203; ref. 11). Expression of three genes, miR-92, miR-20, and miR-18, was inversely correlated with the degree of hepatocellular carcinoma differentiation (13). Such results strongly suggest that quantification of miRNAs may be diagnostically useful.

To understand the possible role of miRNAs as putative therapeutic agents, we have to elucidate the consequences of the widespread miRNA dysregulation in cancer cells. In lung cancers, activation of RAS genes by point mutations, identified more than two decades ago, may represent an early event in some tumors. RAS protein is significantly higher in lung tumors than in normal lung tissue whereas let-7 expression is lower in lung cancer cells. This correlation led to the identification of a direct regulation of RAS by the let-7 miRNA family (33). Exogenous delivery of let-7 to the lung might either prevent the formation of lung tumors (from premalignant lesions) or shrink tumors with activating RAS mutations (34).

MiRNAs are natural antisense interactors with players in the eukaryotic survival and cell cycle programs. The overexpression of antiapoptotic protein BCL2 is an important genetic event in human tumorigenesis, including follicular lymphoma, lung cancer, and B-CLL. The mechanism of this activation, except in all cases of follicular lymphomas where a translocation t(14;18) is responsible (35), was unknown. Loss of miR-15a/miR-16-1 in CLL results in BCL2 overexpression and restoration of mir-15/miR-16 in leukemia cells induces apoptosis by directly interacting with BCL2 miRNA (36). These results are encouraging in the light of new promising results on the therapeutic potential of antisense BCL2.

The oncogene c-myc encodes a transcription factor that regulates, via several targets including E2F1 transcription factor, cell proliferation and survival. A feedback regulatory loop in which MYC directly binds and activates the transcription of the cluster miR-17-92 that consequently negatively regulates E2F1 by direct interaction, while c-Myc is directly inducing expression of the E2F1 that in turn induces c-Myc, was recently described (37). This fine molecular dissection of an important cellular pathway has cancer implications, as it was shown that c-myc and miR-17-92 cooperate and such cooperation accelerates B-cell tumorigenesis in a mouse lymphoma model (29). Such results offer a rationale basis for targeted therapy (e.g., using antisense miRNAs against the clustered miRNAs) that will overload the regulatory loop, with the acceleration of the MYC-E2F1 feedback and consequent cell death.

**The “MiRNA Cascade”**: A Model of MiRNA Involvement in Human Cancers

MiRNAs are contributors to oncogenesis, functioning as tumor suppressors (as is the case of miR-15a and miR-16-1) or oncogenes (as is the case of miR-155 or miR-17-92 cluster; Fig. 1; refs. 21, 34). The classic tumorigenesis model postulates alterations in protein-coding oncogenes and tumor suppressor genes. Relatively minor variations in the levels of expression of a miRNA or mutations that affect moderately the conformation of miRNA:mRNA pairing could have important consequences for the cell. The explanation is provided by the large number of targets of each miRNA and the relatively large number of altered miRNAs, making very likely that two or more PCGs from different molecular pathways/interacting pathways are disturbed. The down-regulation of the suppressor miR-15a/miR-16-1 induces overexpression of BCL2 and possibly other genes that may be important for tumorigenesis, whereas the overexpression of oncogenic miR-17-92 cooperates with c-myc in stimulating proliferation. Therefore, the miRNAs may act “in cascade” over several cancer-specific PCGs, which in turn could influence the transcription or function of several other PCGs and noncoding RNAs including miRNAs. If miRNA alterations occur in somatic cells, they could initiate or contribute to tumorigenesis, whereas, if present in the germ-line, could represent cancer-predisposing events. A paradigm for this model is human B-CLL, in which miR-15a and miR-16-1 are located...
in the most frequently deleted genomic region, are down-regulated in the majority of cases, harbor mutations in familial cases, and induce apoptosis in a leukemia model by targeting the overexpressed antiapoptotic BCL2 gene. As the puzzle of noncoding RNA involvement in cancer is just starting to be assembled, certainly further unexpected pieces will be identified in the near future.

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