Human Cancers: The Interplay Between Protein-Coding Genes and Non-Coding RNAs

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ABSTRACT

The discovery of non-coding RNAs (ncRNAs) dramatically changed the understanding of cancer mechanisms in the last decade. The ncRNAs interplay with protein-coding genes and their abnormalities represent one the most unexpected and important discoveries in the cancer field. Cancer initiation, progression and dissemination causally involve the effects of small regulatory ncRNAs named microRNAs, mainly due to deregulation of expression of cancer protein coding genes. miRNAs can act as oncogenes (activating malignant potential) or tumor suppressors (inhibiting malignant potential) directly on the tumor cells or via communication with tumor microenvironment cells. Understanding the roles of miRNAs and other ncRNAs in malignant cells uncovers a new layer of protein coding and non-coding gene regulation; furthermore, provides new markers for early diagnosis and improved prognosis, as well as novel therapeutics for cancer patients. Herein I will expose what is known about the miRNA function and describe examples and the challenges for clinical use of miRNAs in the near future.

Key words: microRNAs, oncogene, tumor suppressor, cancer, diagnosis, therapy

microRNAs are short RNAs involved in physiologic and pathological processes

MicroRNAs are defined as short non-coding RNAs (ncRNAs) of about 19 to 23 nucleotides (nt) in length, that are not translated in peptides but regulate protein coding gene expression at the posttranscriptional level (1). Precursor microRNAs in the form of hairpin loop structures, located in the nucleus, produce mature microRNAs that act at the cytoplasm level. The binding takes place at the target messenger RNAs (mRNAs) 3'untranslated regions (UTR), coding sequences or 5'UTR (mRNAs) (2). This leads to degradation of mRNA or translation inhibition with consequent target protein expression reduction. It is estimated that miRNAs regulate most part of the human genome, both protein coding as non-coding; recently, it was found that miRNAs could bind and block
the function of longer non-coding RNAs (3). The importance of microRNAs in all physiological or pathological cellular processes is supported by the fact that their structure is highly conserved among orthologous species. Cell cycle regulation, immune system functionality, apoptosis or cell death, cellular aging, differentiation, metabolism and neuronal patterning are all regulated by these short ncRNAs (4,5).

**microRNAs alterations are identified in all human cancers analyzed to date**

Normal levels of expression of mature and/or precursor miRNAs in normal cells versus the abnormal malignant cells represent the main mechanism of microRNAoma (the full spectrum of microRNAs from human genome) alterations (table 1) (6). This is due to numerous reversible or irreversible altered mechanisms, such as the epigenetic regulation of miRNA expression, microRNAs mutated loci, the location of miRNAs at aberrant cancer genomic regions, or defects in miRNA processing proteins including mutations in Dicer (an endoribonuclease involved in the production of mature miRNAs) or Exportin 5 (a protein involved in transport of pre-microRNA out of the nucleus) (7,8).

In 2002, Calin and colleagues reported for the first time miRNAs abnormalities in cancer: mir-15a and mir-16-1, that are located at the frequently deleted sites of the frequent deleted region on chromosome 13q14.

### Table 1 - Examples of oncogenic or suppressor microRNAs

<table>
<thead>
<tr>
<th>Human MicroRNAs</th>
<th>Examples of clinical correlations</th>
<th>Examples of molecular mechanisms</th>
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<tbody>
<tr>
<td>let-7 family [TS]</td>
<td>Let-7b is reduced in leukemia (ALL, CLL), ovary, prostate, liver, brain cancers and correlates with poor prognosis; Lets-7i expression is reduced in breast, brain cancers and associated with bad prognosis; let-7i affects chemotherapy potency</td>
<td>Represses cell proliferation and growth let-7i promotes angiogenesis Targets: CCND1, CDC25a, CDC34, CDK6, Dicer, HMG2, HOX9, ITGB3, MYC, RAS, TLR4</td>
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<tr>
<td>miR-15a, miR-16-1 cluster [TS]</td>
<td>Downregulated in CLL and associated with good prognosis</td>
<td>Induces apoptosis in leukemia cells miR-16 regulates cell cycle by downregulating G0/G1 proteins Targets: BCL2, CCND1, CDK6, CDC27, HMG2, MCL1, MYB, VEGF, WNT3A</td>
</tr>
<tr>
<td>miR-17, miR-18a, miR-19a, miR-20a, miR-92a, miR-17-92 cluster [OG]</td>
<td>High levels of miR-92a identified in leukemia, (CLL, ALL) colorectal and ovary cancers. Associates with poor prognosis.</td>
<td>mir-17, mir-18a, mir-19a, mir-20a, mir-19b-1 increase tumor growth and tumor vascularization; mir-20a is anti-apoptotic; Targets: AIB1, AML1, BIM1, E2F1, E2F2, E2F3, HIF-1A, PTTEN, TGFBR2, TSP1</td>
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<tr>
<td>miR-21 [OG]</td>
<td>Overexpressed in leukemia (CLL), liver, breast, colon, lung, pancreas, prostate, stomach, colorectal, brain, ovary, tongue, thyroid, uterus, head and neck cancers. Poor prognosis, associates with fludarabine refractory CLL</td>
<td>miR-21 knockdown induces apoptosis in glioblastoma miR-21 induces invasion, metastasis in colorectal cancer Targets: BCL2, CDC25A, MASPIN, PDCD4, PTTEN, TPS1, RECK, RASA1</td>
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<tr>
<td>miR-34 family [TS]</td>
<td>Downregulated in leukemias and solid cancers and expression controlled by TP53 Low expression correlates with poor prognosis in leukemias</td>
<td>miR-34a induces downregulation of E2F in colon cancer Targets: BCL2, CCND1, CCNE2, CDK4, CDK6, c-MYC, DLL1, E2F3, HMG2, MET, MYC, N-MYC, Notch1, SIRT1</td>
</tr>
<tr>
<td>miR-155 [OG]</td>
<td>Overexpressed in leukemia (CLL, AML), liver, breast, pancreas, lung, head and neck, thyroid, tongue carcinomas</td>
<td>Pre-B cell proliferation, lymphoblastic leukemia/high-grade lymphoma in miR-155 transgenic mice Targets: AGTR1, AID, FOXO3A, IKKBE, SHIP-1, SOCS1, TPS3N1P1 Modulates mismatch-repair genes</td>
</tr>
<tr>
<td>miR-181 family [TS or OG]</td>
<td>Overexpression of miR-181a is reported in pancreas, thyroid cancers, while downregulation in brain cancers. High miR-181a correlates with short interval from diagnosis to therapy in CLL</td>
<td>MYCN regulates transcription of miR-181 cluster Targets: HOX11, TCL1</td>
</tr>
</tbody>
</table>

Note – TS – tumor suppressor role; OG – oncogene role; the gene symbols are as in NCBI at http://www.ncbi.nlm.nih.gov/. AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia;
The measurement of miRNAs in tumor tissues, plasma, serum and other body fluids represent a new exploratory road for noninvasive biomarkers in cancer (15,16). MicroRNA expression has been shown to forecast the clinical progression of cancers and other diseases. In CLL, both miR-29c and miR-223 are down regulated in patients who are predicted to have a poor prognosis with a shorter survival, while another important therapeutic candidate, miR-155 has been shown to be upregulated in the same category of patients. Also, patients with other hematological diseases like acute myeloid leukemia who present with high miR-191 expression have been reported to have a shorter survival time. Nevertheless, metastasis can be detected by using as biomarkers serum microRNAs known to influence many biological processes and secondary tumor development at additional locations in the body. The concerns about the high stability of miRNAs are mostly disproved by the findings that serum and plasma processing in severe conditions that would normally degrade most RNAs (such as boiling, extreme pH levels, or extended storage) keep the short RNAs unaltered (16). As an example, serum miR-21 levels were lower in hormone-refractory prostate cancer patients who responded to docetaxel-based chemotherapy versus those with resistant disease. Downregulation of let-7a, miR-17, and miR-34 family was correlated with sensitivity to 5-fluorouracil, adriamycin, or cyclophosphamide, all commonly used in various chemotherapy regimens (13). The use of miRNA biomarkers is not restricted only to cancer: for example, the same miR-155 and miR-223 have been implicated in Rheumatoid Arthritis (17). It was also demonstrated the miRNA aberrant patterns in cardiac hypertrophy and their roles analyzed, including that of miR-21, which is also one of the most deregulated miRNAs in cancer, suggesting common miRNA pathways involved in signaling pathways shared by both abnormal states (18).

microRNA therapeutics available for cancer patients

Contrary to chemotherapy, antisense oligonucleotides, small interfering RNAs, or small molecules, there is one major advantage of using miRNAs: they can target multiple genes from the same pathway significantly reducing the potential development of resistance due to multiple mutations in various genes from that specific pathway. For instance, miR-15a and miR-16-1, both with reduced expression in CLL, have two anti-apoptotic targets, the oncogenic messenger RNAs for MCL1 and BCL2. Targeting oncogenic miRNAs with anti-miRNAs or antagoniRs, or restoring the tumor suppressor miRNA levels by using miRNA mimics, although not the “universal panacea” for any type of cancer, could represent in
the near future valid therapeutic options for specific categories of patients (19,20).

In the first miRNA-targeting therapy to reach clinical trials in humans, antagoniR-122 (named Miravirsen) was well tolerated by individuals with of hepatitis C (HCV) infection, a risk factor for developing hepatocellular carcinoma. The use of this antago-miR revealed mild side effects such as diarrhea or headache, being generally well tolerated by patients. Importantly, individuals treated with Miravirsen displayed a significant dose-dependent reduction in HCV RNA levels without any signs of viral resistance (21). A liposome-formulated mimic of the tumor suppressor miR-34a, called MRX34, in a Phase I clinical trial in patients with advanced solid tumors showed manageable toxicity profiles and strong evidence of activity in hepatocellular carcinomas, renal cell carcinomas and melanomas. Molecular analysis showed dose-dependent repression of miR-34a target oncogenes, including BCL2, CTNNB1 HDAC1, and FOXP1 in the tumors from the treated patients (19).

**PERSPECTIVES**

MiRNAs were discovered in 1993 and rapidly became an exciting topic of research during the last decade, with the number of published studies growing exponentially. miRNAs and other longer ncRNAs are involved not only in cancer-altered pathways but also in many other deadly diseases such as sepsis (22). Variations of the miRNome have been documented in cancer cells with respect to the normal cell counterpart. Similarly to microRNAs, other non-coding RNAs (such as circular RNAs or long intergenic non-codingRNAs) (23,24) appear deregulated intumors. miRNAs and other ncRNAs have only recently been identified as new diagnostic and prognostic biomarkers for cancer evolution, and miRNAs based cancer therapy represents a treatment option already in medical practice that has to be tested for safety and efficacy.

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**REFERENCES**


