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Clinical Implications of MicroRNAs in Cancer

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Abstract

MicroRNAs (miRNAs), which are endogenously produced, non-coding RNAs, serve as micromanagers by negatively regulating gene expression. MiRNAs are implicated in several biological pathways, including development of neoplasia. Since altered miRNA expression is implicated in the pathobiology of various cancers, these molecules serve as potential therapeutic targets. The possibility of using miRNA mimics to restore levels of aberrantly down-regulated miRNAs or miRNA inhibitors to inactivate over-expressed miRNAs shows promise as the next generation of therapeutic strategies. Manipulation of miRNAs offers an alternative therapeutic approach for chemo- and radiation-resistant tumors; likewise, miRNA expression patterns can be used for diagnosis and to predict prognosis and efficacy of therapy. The following is an overview of the biogenesis and mechanisms of action of miRNAs, platforms used to measure miRNAs, and the clinical implications of miRNAs in cancer.

Keywords

assay platforms; biomarkers; cancer; clinical outcomes; miRNAs

MicroRNAs and their mechanisms of action

MiRNAs are naturally occurring, non-coding ribonucleic acids (RNAs) that are 18–25 nucleotides in length. They represent a highly conserved evolutionary system that is involved in the temporal and tissue-specific regulation of gene expression at the post-transcriptional and post-translational levels, and they function by binding to target messenger RNAs (mRNAs) involved in cellular differentiation, growth, development, proliferation and apoptosis (Garzon et al. 2006; Perera et al. 2007). Although miRNAs have been found in both plants and animals, the sequence conservation is limited to within kingdoms, as there is no report of any miRNA gene that is conserved between plants and animals (Millar et al. 2005). Altered expression of miRNAs can arise from methylation, mutation, or deletion of miRNA-encoding genes, which may be located near regions that are

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frequently deleted or amplified in cancer (Calin et al. 2004; Caldas et al. 2005). MiRNAs interact with potential oncogenes and tumor suppressors. To date, more than 1500 miRNA sequences have been characterized in humans (the microRNA database is at www.mirbase.org).

In some cases, miRNAs increase gene expression. Most research highlights regulation in which miRNAs decrease the amount of mRNA or protein; however, there is evidence that miRNAs can also increase the amount of mRNA or protein. The tumor necrosis factor α (TNF α) AU-rich element (ARE) recruits *miR-369-3* to mediate translation up-regulation. Base-pairing of *miR-369-3* is required to recruit the translation activating AGO 2-fragile-X mental-retardation-related protein 1 (AGO2-FXR1) complex, and a reporter mRNA containing the TNF α ARE activates translation in cells in response to quiescence via miRNA target sites in the ARE (Vasudevan et al. 2007; Vasudevan et al. 2007; 2008). Current approaches generally neglect this possibility and are restricted to miRNAs negatively regulating mRNAs. Apart from the direct interaction of an miRNA and an mRNA target, an miRNA can also act on an intermediate regulatory molecule that then affects a functional target mRNA (Engelmann et al. 2012). When intermediates are involved, increased miRNA levels may lead to repression or elevation of gene expression levels.

MicroRNAs as biomarkers

As biomarkers, microRNAs can be objectively measured and evaluated as indicators of normal biological or pathogenic processes and of pharmacologic responses to therapy (Hulka BS 1990). Biomarkers can reflect the entire spectrum of disease, from the earliest manifestations to the terminal stages, and are therefore useful in the investigation of the natural history and prognosis of a disease (Perera et al. 2000). Biomarkers have utility in screening and in diagnostic testing for diseases, and can also provide insight into disease progression, prognosis, and response to therapy. With respect to cancer, miRNAs may serve as biomarkers in the early detection or diagnosis of cancer, allowing prediction of patient prognoses and the efficacy of therapy (Manne et al. 2010).

MicroRNAs as biomarkers for early detection and progression of cancers

Early detection is a key measure to reduce the mortality and to improve the prognosis of cancer (Richart 1995; Kalager et al. 2009; He et al. 2011). Identification of sensitive, specific, and non-invasive biomarkers for detecting tumors at an early stage would have useful applications in cancer prevention and therapy. Circulating miRNAs are present in cell-free forms in body fluids such as plasma and serum (Lawrie et al. 2008; Olivieri et al. 2012; Zen et al. 2012; Shen et al. 2013). These miRNAs have distinct expression profiles in cancer and are highly stable in serum and plasma, making them potentially useful for early cancer detection, for they are readily detected and easy to access, which gives them the potential to serve as non-invasive biomarkers (Gorur et al. 2012; Cuk et al. 2013).

Circulating miRNAs have also been detected in urine and saliva. Among 157 miRNAs being analyzed, bladder cancer patients had higher expression of miR-126 and miR-182 in their urine compared to urine samples from healthy volunteers (Hanke et al. 2010). The isolation

and characterization of expression of miRNAs obtained from saliva showed promise as future biomarkers for the diagnosis and prognosis of salivary gland pathologies (Michael et al. 2010). In particular, expression levels of miR-125a and miR-200a are lower in the saliva of patients with oral squamous cell carcinoma than in control subjects, suggesting that salivary miRNAs can be used for detection of oral cancer (Park et al. 2009).

Several profiling studies have been performed on tissues from various types of cancer, including hepatocellular carcinoma, pancreatic cancer, colon cancer, breast cancer, lung cancer, and glioblastoma (Ciafre et al. 2005; Iorio et al. 2005; Cummins et al. 2006; Murakami et al. 2006; Roldo et al. 2006; Yanaihara et al. 2006). Results of these studies have established that different sets of miRNAs are aberrantly expressed in tumors versus their corresponding normal tissues.

In one study, plasma was taken from patients with early-stage breast cancer at the time of diagnosis. From these samples, RNAs were extracted and profiled for miRNAs, and these miRNAs were compared to the same miRNAs extracted from healthy control individuals. MiR-148b, miR-376c, miR-409-3p, and miR-801 were up-regulated in the plasma of breast cancer patients, allowing a conclusion that these miRNAs would be of potential use in the development of a blood-based screening test to complement and improve early detection of breast cancer (Cuk et al. 2013). Similarly, miR-195 was over-expressed only in blood samples from 83 breast cancer patients relative to levels in samples from 63 control subjects (Heneghan et al. 2010). Expression of miR-181b is lower in peripheral blood mononuclear cells of patients with chronic lymphocytic leukemia (CLL), and its decreased levels are associated with the disease progression (Visone et al. 2012), suggesting that levels of miR-181b could be used for monitoring the course of this disease.

For gastric cancer, circulating miRNAs in plasma samples from 20 gastric cancer patients and 190 healthy controls were profiled and quantified. Of 740 miRNAs, 14 were down-regulated and 11 were up-regulated in the cancer patients relative to the control group. These results were compared with patient clinical findings to assess the potential of serum miRNAs as biomarkers for early detection of gastric cancer (Gorur et al. 2012). Nine serum miRNAs were over-expressed in the plasma of gastric cancer patients relative to the controls, and miR-1, miR-20a, miR-27a, miR-34, and miR-423-5p were identified as a molecular signature of serum miRNAs that can serve as a biomarker for gastric cancer detection (Liu et al. 2011). The expression levels of these serum miRNAs were correlated to tumor stage and these results indicate that serum miRNAs could potentially be used as a biomarker to indicate tumor progression stages. The clinical applications of miRNAs also were assessed in monitoring progression of gastric cancer. Since, for colorectal cancer (CRC), miR-92 is elevated in the plasma of CRC patients, it can potentially be used as a non-invasive molecular marker for CRC screening (Ng et al. 2009).

Additionally, fecal miRNAs have been evaluated as biomarkers for screening of CRCs. Extracted miRNAs from stool specimens of 29 CRC patients were evaluated and compared to those of 8 healthy volunteers. MiR-21 and miR-106a were over-expressed in samples from patients with adenomas and CRCs relative to amounts in samples from individuals without these neoplastic lesions (Link et al. 2010). The conclusion was that unique and

identifiable miRNA expression profiles could be acquired from stool samples of patients with CRCs. Thus, fecal miRNAs are promising candidates for a noninvasive method for CRC screening.

MicroRNAs as biomarkers of prognosis

New prognostic molecular markers are needed to improve the clinical outcomes for cancer patients. Profiling of miRNAs has become increasingly relevant in the prognosis of various cancers. For patients with acute myeloid leukemia (AML), an miRNA signature composed of 12 miRNA probes is associated with disease-free survival (Marcucci et al. 2008); and low expression of miR-181a and miR-181b has been associated with shorter overall survival and treatment-free survival in patients with CLL (Zhu et al. 2012). High levels of miR-326/miR-130a and low levels of miR-323/miR-329/miR-155/miR-210 are associated with increased overall survival of patients with glioblastomas, and high miR-326/miR-130a and low miR-155/miR-210 are associated with increased progression-free survival (Qiu et al. 2013). MiR-375 is frequently down-regulated in patients with esophageal squamous cell carcinomas and is associated with advanced clinical stage, metastasis, and poorer outcomes (Li et al. 2013). Likewise, high expression of miR-155 and low expression of let-7a-2 correlates with poor survival for patients with lung adenocarcinomas (Yanaihara et al. 2006).

The prognostic significance of miR-372 in human hepatocellular carcinoma (HCC) was investigated, and a correlation between up-regulation of miR-372 and advanced TNM stage of HCC patients was identified. Also, higher miR-372 expression was associated with poorer recurrence-free survival and overall patient survival in these patients (Gu et al. 2013). MiRNAs can also be used in determining the prognosis of ovarian carcinoma. Ovarian cancer patients having low let-7a-3 promoter CpG island methylation have worse survival compared to those with high methylation (Lu et al. 2007), and, when they are expressed at low levels, the miR-200 family cluster predicts poor survival (Hu et al. 2009). High expressions of miR-372, miR-10b, miR-21, miR-200c, and miR-143 in CRCs are each associated with a short survival times (Xi et al. 2006; Schetter et al. 2008; Altomare et al. 2012; Nishida et al. 2012; Yamashita et al. 2012), whereas down-regulation of miR-106a is associated with shorter survival (Diaz et al. 2008). Our unpublished results demonstrate that over-expression of miR-181b in CRCs is associated with decreased overall survival of Stage III patients, particularly for African Americans (Bovell et al. unpublished). Similarly, higher expression of miR-21 is associated with an increase in the stage of renal cancer and with shorter survival of these patients (Zaman et al. 2012). High expression of miR-21 correlates with lower overall survival of patients with breast cancer (Lee et al. 2011). In contrast, reduced expressions of let-7b and miR-205 are associated with poor survival for patients within luminal and ductal breast tumors, respectively (Quesne et al. 2012). These reports suggest that miRNAs have clinical utility in cancer and will aid in designing therapeutic strategies based on the aggressiveness of cancers.

MicroRNAs as biomarkers of prediction for response to therapy

Although chemotherapy is widely used for the treatment of cancer, drug resistance is a major obstacle to its success (Longley et al. 2006). Through mutations or various genetic alterations, tumors become unresponsive to treatment (Roberti et al. 2006). The possibility of using miRNA mimics to restore levels of aberrantly down-regulated miRNAs or using miRNA inhibitors to inactivate over-expressed miRNAs shows promise for the next generation of therapeutic strategies (Si et al. 2007; Felicetti et al. 2008; Mercatelli et al. 2008; Wickramasinghe et al. 2009; Yang et al. 2009). Tumor chemo-resistance to certain types of drugs may be influenced by miRNA regulation, and the response of cancer cells to chemotherapeutic drugs can be modulated by miRNAs (Meng et al. 2006; Nakajima et al. 2006; Si et al. 2007; Song et al. 2010; Jiang et al. 2011).

Inhibition of miR-21 and miR-200b in cholangiocarcinomas increases gemcitabine-induced cytotoxicity to these cells (Meng et al. 2006). Similarly, for breast cancer, silencing of miR-21 is associated with increased sensitization of MCF-7 cells to topotecan (Si et al. 2007), a chemotherapeutic agent commonly used to treat ovarian cancer. Down-regulation of miR-181b and miR-21 in gastric cancers is associated with increased survival of these patients treated with the 5-fluorouracil (5-FU)-based antimetabolite S-1 and doxifluridine-based regimens (Jiang et al. 2011). Various individual miRNAs appear to have functions in chemotherapeutic responses in CRCs as well. CRC patients with lower levels of let-7g or miR-181b have increased responses to treatment with S-1 (Nakajima et al. 2006), and inhibition of miR-31 increases the chemo-sensitivity of CRC cells to 5-FU (Wang et al. 2010). Over-expression of miR-215 decreases sensitivity of CRC cells to methotrexate (MTX) and tomudex (Song et al. 2010), and, for CRC cells, over-expression of miR-140 increases chemo-resistance to 5-FU and MTX (Song et al. 2009). In the latter case, upon knockdown of miR-140, the resistant phenotype reverts, and chemo-sensitivity to 5-FU is restored in CRC stem cells. This suggests that, in CRCs, miR-140 is a target for developing therapies to overcome chemo-resistance.

Further, miRNAs can sensitize tumors to chemotherapy (Zaman et al. 2012). In CLL cells, the over-expression of miR-15 and miR-16 counteract resistance to tamoxifen. These miRNAs act as tumor suppressors by negatively regulating the anti-apoptotic molecule, BCL-2, and sensitize CLL and breast cancer cells to tamoxifen (Cimmino et al. 2005; Cittelty et al. 2010). In CRCs, increased expression of miR-143 is associated with decreased viability and increased cell death after exposure to 5-FU. The sensitivity of colon cancer cells to 5-FU is increased upon over-expression of miR-143, probably by action through key proteins in pathways involved in the regulation of cell proliferation, death, and response to chemotherapy (Borrallho et al. 2009). Over-expression of miR-34a indirectly increases sensitivity of CRC cells to 5-FU through SIRT1/E2F3 signaling pathways (Akao et al. 2011), and over-expression of miR-192 in CRC cells increases their sensitivity to MTX through inhibition of dihydrofolate reductase (DHFR) (Song et al. 2008).

There is now an emerging idea that single-nucleotide polymorphisms (SNPs) in microRNAs and microRNA target sites have implications for prognosis and prediction of response to chemotherapy of cancer patients. The SNP 829C->T in the 3'UTR of the DHFR gene is

located next to the target site of miR-24, preventing its binding and resulting in an increase in DHFR expression (Goto et al. 2001; Mishra et al. 2007). In turn, this over-expression of DHFR causes DG44 ovarian cancer cells to become resistant to treatment with MTX (Mishra et al. 2007). In metastatic CRCs, an SNP in miR-26a-1 is associated with disease response to irinotecan-based chemotherapy (Boni et al. 2011), and six SNPs, either inside miR-423 or within its binding site of mRNA targets, are associated with disease progression and mortality in prostate cancer patients receiving androgen-deprivation therapy (ADT) (Bao et al. 2011). As determined by this study, SNPs inside miRNAs and miRNA target sites may be independent predictors of clinical outcomes for prostate cancer patients following ADT. Understanding how these SNPs in miRNAs or in their binding sites of target genes contribute to the increasing resistance or sensitivity of cells to chemotherapeutic agents can help tailor individual therapeutic interventions and result in better prediction of risks.

Once identified, there are several approaches to modulate pharmacologically relevant miRNAs in cancer to establish their functions or for future applications in therapy. One approach is to use drugs, including small molecules that modulate miRNA expression by either mimicking the expression of tumor-suppressor miRNAs that have been down-regulated in cancer or by introducing synthetic miRNAs complementary to the miRNAs of interest (“antagomiRs”) to inhibit oncogenic miRNAs that are over-expressed in tumors (Garzon et al. 2010). MiRNA-21 is over-expressed in many solid tumors and hematological malignancies, and an azobenzene compound that could inhibit miR-21 expression and elicit anti-tumoral effects in cells has been identified (Gumireddy et al. 2008). Small molecule inhibitors of miR-122 reduce hepatitis C virus replication in liver cells, and small molecule activators of miR-122 inhibit the viability of HCC cells (Young et al. 2010). These studies support the concept that inhibitors and activators of specific miRNAs have potential in therapeutic applications. A summary of all the aforementioned diagnostic, prognostic, and therapeutic miRNAs in relation to cancer is provided in Figure 1.

Future perspectives and conclusions

Even though many reports have declared the discovery of biomarkers for cancer diagnosis, the United States Food and Drug Administration (FDA) has only approved less than one new protein diagnostic marker per year for implementation in routine clinical diagnoses (Anderson et al. 2002). Cancer biomarkers must be sensitive enough to identify individuals with disease and specific enough to exclude healthy individuals. However, since no currently used biomarker is 100% specific and sensitive, there is a need for use of more than one, or perhaps a panel of biomarkers, in molecular screening for diseases (Manne et al. 2005). For miRNAs to be used as cancer biomarkers, they have to meet the general requirements of being measureable, reliable in assessing progression of disease and patient prognosis, and capable of predicting drug efficacy (Biomarkers Definitions Working 2001).

A surrogate endpoint is an outcome observed prior to a health outcome of interest (called the true endpoint), such as disease incidence, mortality due to disease, or disease relapse or recurrence, and is used to make conclusions about the effect of intervention on the true endpoint (Manne et al. 2005). If miRNAs are to be utilized as intermediate endpoint

biomarkers, chemotherapeutic or chemo-preventative agents should be able to modify these miRNAs; and altering the expression of an miRNA should result in a corresponding change in the disease rate (Wagner et al. 2002). Therefore, miRNAs can potentially increase the quality and efficiency of therapeutic interventions.

Studies to identify miRNAs as cancer biomarkers are needed, but they do not come without challenges. One such challenge is the heterogeneous nature of cancer, which produces a variety of genotypes and phenotypes that affect patient responses to treatment. Next, validation studies involving large, independent cohorts of patients from various institutions are required to establish miRNAs as clinical cancer biomarkers. These studies should involve clinical trials to evaluate risk-benefit associations in assessing drug effects and measuring efficiency in drug development. Moreover, considerations should be taken when deciding which miRNAs are worth validating clinically, as conducting clinical trials for potential biomarkers are laborious and costly. Since miRNAs are involved in various oncogenic signaling pathways, another challenge is to identify potential miRNA targets and the underlying downstream mechanisms that contribute to tumorigenesis and disease progression. In the future, strategies to manipulate or modify miRNAs may be used in conjunction with, or in lieu of, current anti-cancer therapies to increase sensitivity in drug-resistant tumors. There is already interest in preventing tumor recurrence by using miRNA-based strategies to control latency and stemness of cancer stem cells (Aslam et al. 2012). The emerging field of miRNA-based gene therapy has considerable potential, but much work remains to be done in developing effective miRNA-specific delivery systems and in assessing down-stream side effects that should be avoided.

In conclusion, recent advances suggest that miRNAs have potential clinical implications, and future efforts in obtaining a more complete picture of the role of miRNAs and their applications in cancer management are warranted.

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Figure 1.
Summary of diagnostic, prognostic, and therapeutic miRNAs in cancer.