MicroRNAs in personalized cancer therapy


MicroRNAs (miRNAs) are small endogenous noncoding single-stranded RNAs. They critically regulate the post-transcriptional activity of several key physiological and pathological cell processes including cancer. Through their transcriptional regulatory functions, miRNAs control tumor proliferation, invasion and metastasis. The expression of miRNAs is altered in malignancies. It could be either upregulated or downregulated depending upon the role of a particular miRNA in the pathogenetic development of the tumor. The upregulated miRNAs exert an ‘oncogenic’ effect leading to tumor proliferation and metastasis. The downregulated miRNAs have ‘tumor suppressor’ effects. Recent studies have demonstrated that miRNAs have a role in the early diagnosis, prognosis and treatment outcome assessment of cancers. Every tumor has specific miRNA alterations, i.e. some are overexpressed and others are downregulated. These altered miRNAs can be used as a tumor-specific ‘signature’ for potential clinical use in improving the accuracy of diagnosis, determining prognosis and as therapeutic targets for therapy. Specific miRNAs can be targeted using oligonucleotide sequences corresponding to the altered miRNAs. These are referred to as ‘antagomirs’. Depending upon the miRNA alterations in the tumor of an individual patient, one could design targeted therapies for personalized medicine in patients. Hence, miRNAs have an immense role in personalized cancer therapy.

Conflict of interest
The authors have no conflicts of interest. They do not have any financial and personal relationships that might bias their work. They are involved with the design, writing of the paper and in the decision to submit the report for publication.

MiRNAs: the novel molecules
MicroRNAs (miRNAs) are small endogenous noncoding RNAs that critically regulate both pathological and physiological processes within cells (1). Although these are tiny molecules of about 19–25 nucleotides, they play important roles in many vitally important cellular processes including cell differentiation, proliferation and apoptosis (2).

During physiological fetal development, they assist in the differentiation of the pluripotent fetal stem cells into the three germ layers: endo-, meso- and ectoderm. This process leads to the normal development of the body’s organ system. The endoderm develops into lining of the gastrointestinal tract and lungs; mesoderm develops into the mature muscle, bone and blood; and the ectoderm develops into the skin. Specifically, messenger RNA (mRNA) for Lin28 is a promoter of pluripotency and it prevents the accumulation of miRNA let-7 (1). Similar to the differentiation of the fetal stem cells, which leads to normal development of the fetus, recent studies indicate that cancers also evolve through differentiation of cancer stem cells (CSCs) into specific tumors acquiring a specific lineage, e.g. squamous cell carcinomas or adenocarcinomas (2). These undifferentiated CSCs have self-renewal and tumorigenic potential with invasive and metastatic propensities (3). Similar to the normal
Table 1. List of upregulated or downregulated microRNAs (miRNAs) involved in different tumor types

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Upregulated miRNA</th>
<th>Downregulated miRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>miR-21, miR-221, miR-222, miR-17-92, miR-155</td>
<td>miR-200 family, miR-342, let-7 family</td>
</tr>
<tr>
<td>Colon</td>
<td>miR-21, miR-31, miR-378</td>
<td>miR-362, miR-375, miR-200 family</td>
</tr>
<tr>
<td>Leukemia</td>
<td>miR-155, miR-17-92, miR-21, miR-125b, miR-93, miR-196b, miR-223, miR-34a</td>
<td>miR-15, miR-16</td>
</tr>
<tr>
<td>Lung</td>
<td>miR-192, miR-424, miR-98, miR-155</td>
<td>Let-7 family, miR-200 family, miR-194, miR-212</td>
</tr>
<tr>
<td>Pancreas</td>
<td>miR-21, miR-17-92, miR-155, miR-221, miR-222</td>
<td>Let-7 family, miR-200 family, miR-146a, miR-143</td>
</tr>
<tr>
<td>Prostate</td>
<td>miR-20a, miR-21, miR-221, miR-222</td>
<td>Let-7 family, miR-628, miR-200 family, miR-7, miR-29C</td>
</tr>
<tr>
<td>Thyroid</td>
<td>miR-155, miR-21, miR-222, miR-146b, miR-34a</td>
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ahttp://www.ncbi.nlm.nih.gov/pubmed/

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Fetal development, the miRNAs also regulate the tumor development from CSCs, e.g. let-7 and Lin28 regulate the tumorigenic capabilities of the cancer cells (4). Nowadays, tiny noncoding RNAs, called miRNAs, have captured the spotlight in molecular biology with highlights such as their involvement in DNA translational control, their role in mRNA and protein expression levels, and their ability to reprogram molecular signaling pathways in cancer. Realizing their pivotal roles in drug resistance, they emerged as diagnostic targets orchestrating drug response in individualized therapy. It is not premature to think that researchers could have the US Food and Drug Administration (FDA)-approved kit-based assays for miRNA analysis in the near future. We believe that miRNAs are becoming ready for prime time.

Mechanism of action of miRNAs

The action of miRNAs in cancers is mitigated by the regulation of gene expression via translational repression and transcript degradation. They act via the regulation of miRNAs. The 5’end of miRNA binds to complementary regions in the 3’ untranslated region (UTR) of the target miRNAs, leading to either degradation of mRNA or inhibition of its translation to proteins, further affecting the cell signaling pathways. However, the regulatory role of miRNA in mRNA stability and translation into protein is a complex biological process, which is not restricted through the binding of miRNA only in the 3’-UTR of the mRNA.

Human miRNAs: types

More than 2555 miRNA sequences have been recognized in humans. Of these, some are aberrantly expressed in malignancies (4). The altered miRNAs may have different effects on the tumors as presented in Table 1 (summarized from a search: http://www.ncbi.nlm.nih.gov/pubmed/). They may be oncogenic or tumor suppressor miRNAs. The oncogenic miRNAs are upregulated in cancer, e.g. miR-21 (5), miR-17-92 (2), miR-155 (6), miR-221 (7) and miR-222 (8). Tumor suppressor miRNAs are downregulated in cancers, e.g. let-7 (9) and miR-15 (10, 11).

MiRNAs in tumors

Every tumor thus has a unique combination of miRNAs, i.e. overexpressed oncomiRs and underexpressed tumor suppressor miRNAs (12). This unique combination has the potential to serve as a ‘signature’ for personalizing the therapy of a tumor in an individual patient (13). Tumor-specific miRNAs have potential clinical use as markers for improving diagnostic capabilities, risk stratification and predicting prognosis and as targets for personalized novel therapies. The role of miRNA has been previously published in hepatocellular carcinoma, multiple myeloma and renal cell carcinoma among others (14, 15).

With regard to colorectal carcinoma, Christensen et al. (16) suggested that the increased expression of the particular miRNA-362-3p is associated with cell cycle arrest and tumor growth inhibition. It has been suggested that the increased expression of miRNA is associated with a better prognosis for the patient. Similarly, the miRNAs have also been correlated with dysplasia in bladder cancer. Nordentoft et al. (17) published an analysis of over 600 miRNAs from the tumors of patients with advanced bladder cancer treated with cisplatin and found that 15 miRNAs played a particularly important role in assessing patient’s response to cisplatin treatment, five were related to survival time and three miRNAs were associated with both aspects.

Detection of miRNAs in humans

In this clinical context, we can detect miRNA expression levels in a variety of human specimens including blood, tissues (both fresh and formalin-fixed paraffin-embedded), fine needle aspirates and in almost all human body fluids (10, 13).

The presence of miRNA in various body fluids portends the development of noninvasive testing methods for detection and management of common malignancies. It is especially important that miRNA can be present in especially stable form in serum and plasma, which permits for the measurement of their expression. The stability of these molecules is illustrated by their durability in incubation at room
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temperature to multiple cycles of freeze thawing. Detection of these miRNA can be done by taking specimen samples from patients or through a xenograft model system as well (18). These miRNA signatures can be helpful in improving the accuracy of diagnosis and making an early diagnosis (19).

Clinical potential of miRNAs

As every tumor has a unique miRNA signature, this can be used as a biomarker to identify tumors at the earliest stages of tumor development. This is specifically important because if tumors are diagnosed early, treatment can be initiated immediately and the prognosis would be considerably better than the tumors which are detected late in the course of the disease (20).

In poorly differentiated tumors, there is often a diagnostic dilemma and the exact nature of the tumor is difficult to determine by histomorphological features alone. In such instances, miRNA signatures specific to the tumors hold immense promise in enabling accurate diagnosis (19). Molecular miRNA tumor-specific signatures would be very important for designing optimal primary treatment of malignancies to prevent and eliminate future tumor proliferation and even metastasis. To that end, targeting miRNAs could become a novel prognostic and therapeutic strategy to prevent the tumor proliferation and future development of metastasis (19).

While the initial school of thought aimed to classify miRNA as either oncogenic or tumor suppressive in nature, recent understanding has proved that this belief is not fully accurate. It is now known that a single miRNA can have both roles. The advent of miRNA mimic and miRNA silencing molecules has allowed us to modulate miRNA expression in tumors, showing that miRNAs can be used effectively as therapeutic agents. This review will focus on those findings that have provided the rationale for the use of miRNAs as patient-‘tailored’ anti-cancer agents.

miRNAs as therapeutic targets

Emerging evidence clearly suggests that miRNAs may provide attractive, novel therapeutic targets for cancer treatment. They also have the potential to be used as targets for therapy (19). Furthermore, once the metastasis develops, the tumor could be treated with miRNA mimics for inducing its expression for the treatment. The development of miRNA-based prophylactic therapies could serve as personalized medicine against future development of metastatic spread of cancers. These strategies will ultimately improve patients’ quality of life and will also improve the overall survival. That is one of the reasons that miRNAs are gaining momentum in the clinical arena.

Role of miRNAs in therapeutic resistance

The strategy for miRNA-targeted approach would be extremely relevant clinically in cases with therapeutic resistance. It has often been observed that tumors may initially respond to a therapeutic approach; however, the response is transient and the tumor regrows and even metastasizes, leading to a poor prognosis for the patient. Initially, this phenomenon has been ascribed to the death of tumor cells in response to the therapeutic agent. However, CSCs are generally nonresponsive to the treatment. These CSCs then repopulate the tumor cells with cells that no longer respond to the therapeutic agent and even make the tumor more aggressive (4).

In malignancies developing therapeutic resistance, there are alterations in the expression of miRNAs, e.g. miR-192, miR-424 and miR-98 are overexpressed and miR-194, miR-200b and miR-212 are underexpressed in docetaxel-resistant Non-Small Cell Lung Cancer (NSCLC) cells (21). Individual tumors bearing tumor-specific combination of deregulated miRNAs that contribute to therapeutic resistance make miRNAs as attractive therapeutic targets for personalized approach toward overcoming resistance. To this end, miRNAs are being used to restore the sensitivity of drug-resistant cells to chemotherapy and preventing tumor recurrence (22).

It has been found that knockdown of miR-221 and miR-222 sensitizes breast cancer cells to tamoxifen-induced apoptosis (23). In cancer cells, resistant to 5-FU treatment, miR-140 is blocked and re-expression of miR-200 increases the sensitivity to microtubule-targeting chemotherapeutic agents (24).

MiRNAs have been implicated in the prevention of resistance in breast cancer patients receiving chemotherapy treatment with tamoxifen. The presence of miRNA-342 has been established in tamoxifen-susceptible breast cancer cells, and the suppression of this particular miRNA has been associated with the development of resistance to treatment. Thus, it is logical to believe that because of the inverse relationship of miRNA and drug resistance, increasing the expression of miRNA-342, for example, may result in a more successful therapeutic approach in the treatment of breast cancer (25).

Current approach in the management of cancer patients

At present, management of patients with malignancies is based on the identification of tumor morphology. It is mainly the phenotypic expression that identifies which protocol a patient will receive for a given tumor treatment. With rapid developments in the identification and detection of molecular alterations, a new paradigm of patient management is now being offered as discussed below.

Personalized medicine: the new approach

Rapid development in the field of both medicine and molecular biology has led to the discovery of a plethora of novel biomarkers, which hold immense promise in the clinical management of patients diagnosed with cancers. This has specifically been
possible with tests that enable the detection of these biomolecules in routine biological specimens obtained from patients. Recent studies have demonstrated the potential use of these novel molecular targets in early and accurate diagnosis, prognosis and for assessing treatment outcome. These developments have led to a novel approach in patient management: ‘Personalized medicine’. This approach amalgamates the information yielded by molecular alterations with the phenotypic histomorphological expression and clinical factors to outline patient-tailored management for an individual. Most clinical decisions on patient management currently use the ‘one-size-fits-all’ approach. Studies have demonstrated that this approach may not benefit all patients. Not only does all tumors respond in a similar manner but also the patients’ internal milieu is different that is broadly known as tumor microenvironment. The tumor microenvironment has a huge impact on the response or lack thereof to the therapeutic strategies. This results in several deleterious and disabling side effects, which increase the morbidity of the patient, making this generalized approach non-ideal in the 21st century. The personalized approach to treatment overcomes these limitations of the generalized approach. It provides an opportunity to translate the advances of molecular medicine for clinical patient benefit from the research bench to the patients’ bedside. Increased clinical usage of the personalized approach in medicine can impact several aspects of patient management. We can utilize the novel molecular tests to improve diagnostic accuracy, assist in early and rapid diagnosis, predict disease progression and identify risk factors to predict prognosis. It can also improve patient-specific ‘tailored’ therapeutic approaches based on the unique tumor-specific molecular signatures as targets for therapy.

**MiRNAs for Personalized medicine**

There are many targets for personalized therapy, which has shown limited success. However, the personalized targeted therapeutic approach using miRNAs as targets holds immense promise in revolutionizing the patient treatment dogma. In fact, as we embark toward the era of personalized medicine, the therapeutic decision making in clinical oncology is currently undergoing a transition toward personalized pharmaco-therapeutics. Tumors are being treated more by the molecular characteristics than by histo-morphological features alone (13). It is clinically extremely relevant that miRNAs could serve as potential targets for anti-tumor therapy. The expression of miRNAs can be silenced using ‘antagomirs’, or re-expression of miRNAs that are lost in cancers can be achieved by the overexpression of miRNA mimetic.

The miRNA signature associated with human metastatic medulary thyroid carcinoma and other malignancies has been reported, which certainly opens newer avenue for the development of miRNA-targeted therapy. To that end, chemically modified anti-miRNA oligonucleotides that target specific sequence of miRNAs led to the inactivation of the miRNA activity, thereby preventing further proliferation, invasion and metastasis of the tumor.

**MiRNAs as predictors of response to personalized cancer therapy**

Once the treatment protocol is instituted in a patient, the miRNA signatures specific to the tumor would potentially be useful in monitoring response to therapies and to monitor tumor recurrence (20).

**MiRNAs as prognostic markers of personalized cancer therapy**

These molecules are clinically significant as miRNAs also have the potential to be clinically useful as biomarkers as predictors of prognosis. It has been found that miRNAs are either upregulated or downregulated in tumors, and these expression levels correlate with the tumor behavior, thus making them potentially useful for predicting tumor treatment outcomes (26).

As early as the beginning of the last decade, it was first shown by Calin et al. (27) that miRNA levels are downregulated in a majority of patients with chronic lymphocytic leukemia (CLL). Specifically, the miR15 and miR16 are located at chromosome 13q14 and are downregulated owing to the loss of a 30-kb region in CLL patients.

Similarly, overexpression of miR-155 and underexpression of let-7a-2 were positively correlated with a poor survival in lung cancer patients (28). The reduced expression of let-7b in patients with NSCLC has been correlated with increased mortality, whereas miR-155 expression levels did not display clinically significant correlations (29).

Thus, the roles of many different miRNA have been described in a variety of tumors. However, more study is still needed to fully understand the role of miRNA as biomarkers and assessing their roles in personalized cancer therapy.

**Future challenges of miRNAs in personalized medicine**

While the future of miRNA seems extremely promising, certain challenges will need to be addressed before the miRNA-based treatment methods become widely accepted into clinical practice. The involvement of miRNAs has been proposed in a range of often contradictory functions such as tumor suppression and proliferation. Some miRNA can also have multiple phenotypic effects (30). An example of this can be seen in the action of miRNA-15a/16-1 on leukemic cells with 13q deletion. The miR cluster has targets on both the anti-apoptotic BCL2 as well as the pro-apoptotic p53. In leukemic individuals with 13q deletion and a corresponding decrease in the expression of the miRNA, increasing the expression of the miR cluster results in a significant anti-leukemic effect (31).
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This raises the issue of ensuring that miRNA targeting is specific enough to influence cellular function only in the intended manner to avoid adverse consequences, meaning that the miRNA-targeted therapy must be considered in a context-dependent manner. In our recently reported study, we assumed that the changes observed by miRNA alteration are the result of influence on multiple genes (32).

Another issue that requires further understanding is the amount of miRNA expression that needs to be altered to achieve the desired level of cellular response. While inadequate modulation may result in an insufficient anti-tumor response, excess variation in miRNA may result in unintended consequences, especially because one miRNA could target multiple genes (mRNAs), which are also context dependent. Other questions such as the preferred route of drug administration and duration of action must also be taken into account as well while developing the optimal personalized miRNA-targeted cancer therapy (32).

Further understanding may also help us ascertain whether the effect of miRNA is most beneficial as a sole treatment option or if overall prognosis improves via the simultaneous administration of several treatment options. We must ensure that altering the expression of miRNA does not trigger unanticipated results, which may further complicate future therapeutic modalities. With the increasing study of miRNA in the clinical realm, it is reasonable to expect that these issues will be more clearly elucidated because of the enormous potential of miRNA-targeted therapy as a single modality or in combination with conventional cancer therapy.

Conclusions

In summary, since the recent discovery of the role of miRNA in cellular activity, our knowledge on their action has improved tremendously in the last decade. At this time, we have been able to ascertain the role of miRNA in several physiological processes associated with the initiation and development of various solid as well as nonsolid cancers. We have illustrated the role of specific miRNA, such as let-7 and miR-155 as either tumor suppressors or oncogenic molecules, respectively, identified their role in early detection, and in the prognosis and therapy of patients. If the last decade is any indication of scientific breakthrough in miRNA research, the future appears to be extremely promising for the use of miRNAs in the delivery of personalized medicine for improving the overall treatment outcome and survival of cancer patients, although the success of miRNA-targeted therapy looks brighter than ever before.

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