Review

Profiling microRNA expression with microarrays

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The discovery of several types of small RNAs (sRNAs) has led to a steady increase in available RNA databases. Many of these sRNAs remain to be validated and functionally characterized. Recent advances in microRNA (miRNA)-expression profiling of different tissues, stages of development and physiological or pathological states are beginning to be explored using several technological approaches. In this review, these recent advances in miRNA microarray technology and their applications, particularly in basic research and clinical diagnosis, will be summarized and discussed. The methods for miRNA enrichment and probe design and labeling will also be discussed with an emphasis on evaluation of predicted miRNA sequences, analysis of miRNA expression and exploration of the potential roles of miRNA sequences in the regulation of stem cell differentiation and tissue- and time-specific profiling patterns of their target genes.

Introduction

Small RNAs (sRNAs) are a growing class of recently identified endogenous non-protein-coding sRNAs (ncRNAs) 21–35 nucleotides in length. They can be divided into at least six types, including microRNAs (miRNAs), tiny non-coding RNAs (tncRNAs), small interfering RNAs (siRNAs), repeat-associated small interfering RNAs (rasiRNAs), small modulatory RNAs (smRNAs) and Piwi (P-element-induced wimpy testis)-interacting RNAs (piRNA) [1,2]. The miRNAs have been most widely studied [3] and are derived from introns or extrons of protein-coding and non-coding genes – hundreds of miRNAs have been cloned and sequenced [4,5]. Bioinformatics and genetic analysis suggest that these unique miRNA genes are present in the genomes of mouse, humans, *Drosophila*, *Caenorhabditis elegans*, *Arabidopsis*, viruses and fungi and that they are expressed in a regulated manner. Several investigations have demonstrated that miRNAs serve as guide molecules, in that they form base pairs with partially or fully complementary sequences in target mRNAs leading to translational repression and/or mRNA cleavage [6,7]. Moreover, miRNAs have been shown to induce transcriptional silencing, resulting in the modification of DNA and/or chromatin in yeast, plants and animals [8–13]. They can also potentially regulate hundreds of mRNAs. Some miRNAs have been shown to affect stem cell differentiation, organ development, cell death, phase change, signaling, disease, cancer and the response to biotic and abiotic environmental stresses [3,14–16]. Furthermore, many miRNAs are highly conserved in animals and plants. They have been shown to have unique tissue-specific, developmental stage-specific or disease-specific patterns. These observations imply that each tissue is characterized by a specific set of miRNAs that might be defining features of that tissue [3].

The advent of genomics and the burgeoning amount of genomics data have greatly accelerated studies on structure and function of genes, and significantly altered our understanding of gene regulation. Undoubtedly, microarrays have made important contributions to both basic and applied research, and have the potential to change the practice of medicine to a more personalized endeavor [17–20]. The goal and power of microarray experiments is to survey patterns of mRNA expression by assaying the expression levels of hundreds to thousands of genes in a single assay. However, the profiling of miRNA expression is a relatively new field. Several parameters, including their small size, low abundance and the tissue-, developmental stage- and disease state- specificities in miRNA expression, make the adaptation of array technology to the analysis of miRNA expression challenging [21]. Nevertheless, several methodologies have been adapted for profiling miRNA expression. They include northern blotting with radiolabeled probes [22,23], cloning [24], quantitative PCR-based amplification of precursor or mature miRNAs [25,26], serial analysis of gene expression (SAGE)-based techniques [27], bead-based profiling methods [28,29] and oligonucleotide microarrays [30,21,31]. Several studies demonstrated that miRNA microarrays succeeded in assessing miRNA expression on a global scale and enabled analysis of the expression of hundreds of miRNA genes in a single experiment. miRNA arrays are now being developed to explore the biogenesis of miRNAs, tissue distribution, differential miRNA expression between normal and abnormal states, disease characterization, stem cell development, pathway mapping, mechanisms of action and tumorigenesis [32–34]. Moreover, scientists are also conducting profiling studies that might lead to the use of small RNA arrays in molecular diagnosis of disease and in evaluation of drug efficacy and toxicity. This review

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attempts to focus on the sRNA array technologies and their application in basic and clinical research related to the sRNA 'world' (which includes all types of sRNA with lengths of less than 40 nt).

miRNA microarrays

In contrast to mRNA profiling technologies, miRNA profiling must take into account the difference between mature miRNAs and their precursors, and should also distinguish between miRNAs that differ by as little as a single nucleotide [35]. Moreover, it has been shown that the sequences of mature miRNAs display unequal melting temperatures. These features – specific for miRNAs, present major challenges for sample labeling and microarray probe design. The following section gives critical consideration to capture-probe design, sRNA enrichment and labeling technologies (Figure 1).

Probe design

In gene expression microarrays, either synthetic oligonucleotides or cDNA fragments are used as capture probes. An ideal probe should have high specificity and high affinity. Accumulative data analysis indicates that adenine (A)- and thymine (T)-rich probe sequences frequently exhibit lower hybridization intensities than more stable sequences with high guanine (G) and cytosine (C) content [36]. However, the sensitivity of A/T-rich probe sequences in arrays can be enhanced by the introduction of nucleotide analogs that improve the overall duplex stability [37]. Sequential experiments showed that the substitution of deoxyadenosine (dA) and deoxythymidine (dT) in 10-mer probe arrays with the 2′-O-methyl-2,6-diaminopurine and 2′-O-methyl-5-methyluridine analogs could increase the relative hybridization intensity two- to threefold [38]. The same pitfalls are also found in sRNA arrays. For example, the melting temperatures (Tm) of miRNAs vary between 45 °C and 74 °C. If a specific hybridization temperature (e.g. 55 °C) is set for the entire set of miRNAs, capture probes with lower Tm values will yield lower signals, whereas capture probes with higher Tm values will display impaired nucleotide discrimination and lower specificity. To overcome this problem, Castoldi and co-workers [39] developed a type of locked nucleic acid (LNA)-modified capture probe, which can elevate thermal duplex stability (up to 8 °C per LNA modified base). These results showed that LNA-modified capture probes with

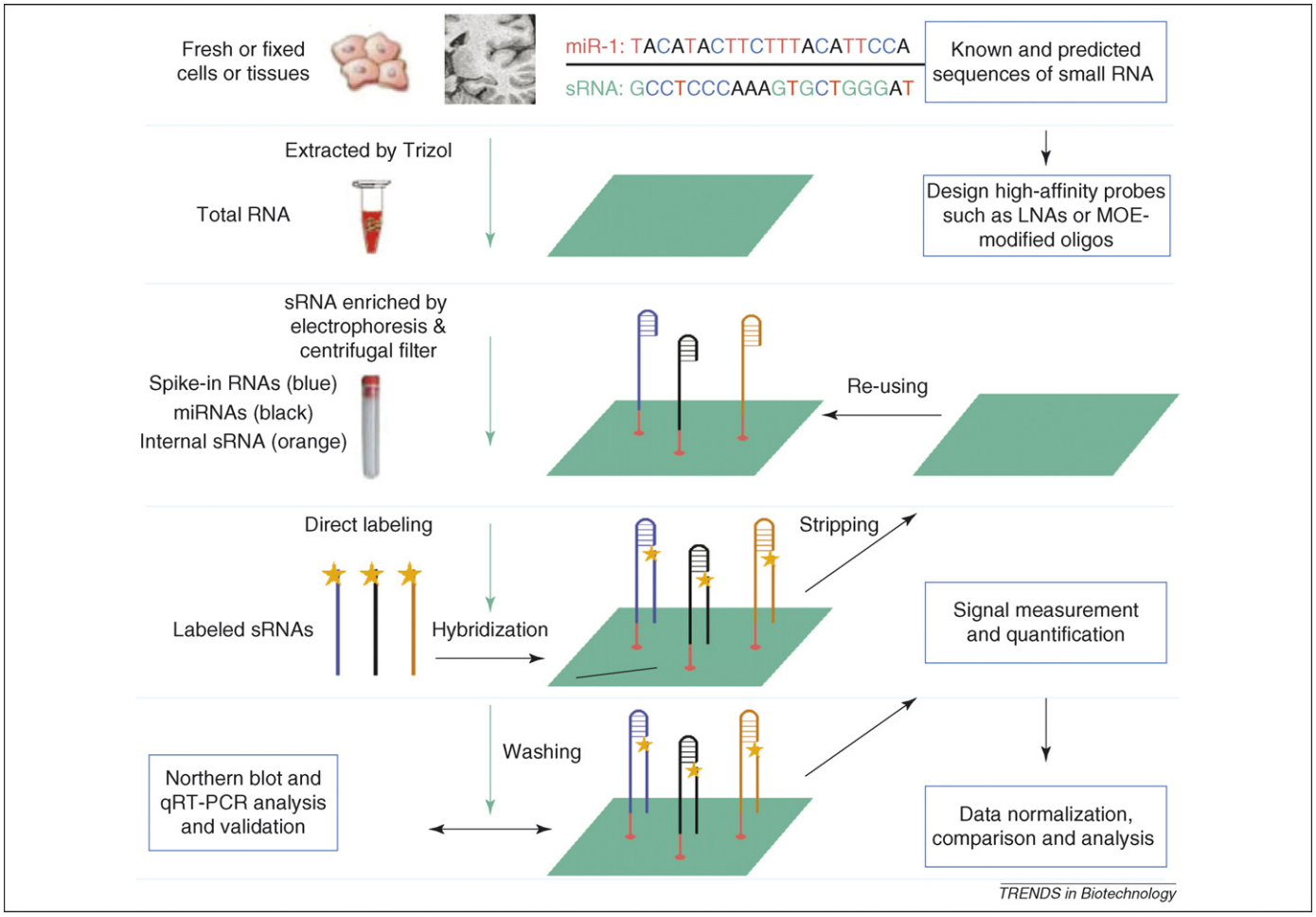

Figure 1. Flow chart for a miRNA array platform. The platform incorporates three major steps: (i) the purification of mature miRNAs from fresh or fixed cells or tissues, separation of sRNA and large RNA (>200nt), enrichment of miRNAs and labeling; (ii) the design of the microarray, including probe design, control design, probe spotting and array hybridization; and (iii) the analysis of experimental results through measurement, quantification, normalization and validation. DNA oligonucleotide probes comprise a universal spacer sequence on their 3′end (red) and the antisense miRNA sequence on their 5′end (orange for spike-in control, blue for internal control and black for specific miRNAs). The miRNAs are labeled with a dye and then directly hybridized to the arrays with the appropriate probes specific for mature miRNAs. The resulting double-stranded fragments can be easily detected. Finally, northern blot and qRT-PCR analysis for some constitutively expressed miRNAs and U6 or tRNA are used for data normalization, validation and comparison.

normalized melting temperatures were more sensitive than DNA capture probes. The researchers speculated that this microarray platform (miChip) could accurately and sensitively monitor the expression of miRNAs without prior need for RNA size fractionation and/or amplification. Similarly, Beuvink *et al.* [40] indicated that the application of 2′-O-(2-methoxyethyl) (MOE)-modified oligoribonucleotides as capture probes could facilitate detection of newly identified validated or predicted miRNA candidates. In addition, to balance melting temperatures of miRNA probes it has been suggested that increasing or reducing the length of probes according to physicochemical traits of the particular miRNA sequence of interest might suffice to provide accurate and sensitive array-based analysis [41]. Moreover, to help distinguish the targeted miRNA from unintended potential targets, Wang *et al.* showed that a hairpin structure incorporated onto the 5′ end of the probe could destabilize hybridization to larger RNAs, such as miRNA precursors [41]. Taken together, the development of these new technologies enables high-affinity hybridizations for all mature miRNAs. Thus, it is possible for miRNA arrays to yield accurate signals that discriminate between single nucleotide differences and identify the expression of known or predicted sRNAs.

In addition to probes that are complementary to the sense and antisense strands of miRNAs, different control probes are also required. They include exogenous and endogenous positive controls and negative controls. These control probes are used to assist normalization and to provide absolute reference points for quality control and quantitative comparison of different microarrays (Box 1). Moreover, common transcripts that are mutually represented among the various microarray platforms and that can be used to compare the expression differences between different arrays should be included in the analysis. This aspect will facilitate cross-platform comparisons of sRNA expression, and potentially lead to robust meta-analyses in clinical miRNA expression studies. In addition to different controls for data quality control, repeated spotting of the same clone on an array has been shown to increase precision of the measurements if the spot intensities are averaged. It can also minimize problems caused by scratches, dust and other mishaps that can contaminate the surface of microarray slides.

Storage, preparation and enrichment of miRNAs

The storage and preparation of miRNAs from samples are crucial for microarray gene expression analysis. Recently, it has been shown that miRNAs within tissues can be kept in a satisfactory state for prolonged periods using formalin. Using LNA-based miRNA array analysis, Xi *et al.* found that the expression of miRNAs from formalin-fixed paraffin-embedded (FFPE) specimens was in good correlation with fresh frozen samples [42], raising the possibility of archiving fixed clinical specimens for further miRNA analysis at a later date. The first step in obtaining miRNA samples is the isolation of RNA from different cells and tissues of different species. Many protocols have been developed for the extraction of high-quality RNA using various in-house and commercial kits and reagents [21]. Generally, reagents used are mainly RNAwiz™ (Ambion Inc., http://www.ambion.com), TRIzol Reagent (Life Technologies Inc., http://www.invitrogen.com) or TRI Reagent (Molecular Research Center Inc., http://www.mrcgene.com) and a silica-based membrane-spin column (RNeasy column, Qiagen Inc., http://www.qiagen.com). These methods can provide high quality RNA for further investigations.

Owing to the miRNA features of low molecular weight and low abundance, miRNA enrichment is an important step for the measurement of expression [21,34,40]. By contrast, the direct use of total RNA to profile miRNA expression can limit sensitivity because the relative abundance of sRNAs in a total RNA sample is ~0.01% [35]. In addition, direct and indirect labeling requires a substantial amount of sRNA, typically between 500 ng and 5 μg. Mature miRNA amplification and high-sensitivity techniques have been developed to overcome this obvious limitation [25,27,28]. In addition, several methods have been established that addressed sRNA enrichment, including the relatively new technology of ion pair reverse-phase chromatography, which has been shown to selectively enrich and separate the less abundant sRNAs from the more abundant higher molecular weight rRNA species. Ambion Inc. developed the mirVana™ miRNA Isolation Kit and a rapid column gel electrophoresis (flashPAGE™ Fractionator), Invitrogen (http://www.invitrogen.com) cre-

Box 1. Control probes

Positive controls

Positive controls are an ideal way to establish parameters that result in successful detection of target RNA. Generally, an oligonucleotide that targets a highly expressing sRNA ensures that the target RNA is expressed at a level that does not fluctuate during the cell cycle.

Negative controls

These are crucial for distinguishing sequence-specific hybridization from non-specific effects in the array experiment. Negative control probes are designed to have no known target in the cells being used or are mutated to create mismatches or blank spots. Alternatively, a negative control could be a miRNA probe that is not expressed in the particular cells being assessed.

Internal controls

These are also extremely useful for evaluating the quality of the microarray. Using a target that is complementary to the immobilized internal control probe or a non-equilibrium dissociation method to determine the dissociation temperature, the quality of the microarray chip can be assessed. The dissociation temperature of the internal control duplex should be equal for all chips used. The use of internal control spots might also help ensure the quality of the data and can help in calibrating the results of an analysis.

External controls (spike-in control)

External controls are RNA molecules that are chemically synthesized *in vitro*. Control RNAs are added to the biological samples of interest in varying concentrations. The spike-ins can be used to measure the gain or loss of signal during each step of the sample preparation process (including total RNA isolation, fractionation of miRNA, labeling and others) and can address process-related losses of miRNA.

Sample size controls

These are used in sRNA arrays. To identify whether mature sRNAs or their precursors bind to the corresponding probes on a slide array, the probes for loop regions of RNAs can be designed and spotted on the array.

Table 1. Methods of miRNA profiling

Method	Merit(s)	Limitation(s)	Refs
miRNA arrays probe design	Low cost; high throughput	Radioactive isotopes	[45]
Nucleotide modification	Tm balance	Needs special nucleotide	[38,39]
RNA length trim	Tm balance, sensitivity (0.2 amol–2 fmol)	Needs labeled donors and RNA ligase	[41]
miChip labeling	Tm balance	Needs LNA	[40]
Indirect	Potential for amplification	Random primers, precursor contamination	[46]
RAKE approach	High specificity	Lower sensitivity	[47]
Cisplatin-based	Chemical label	Hybridization interference	[49]
PCR and Cy3-label	Sample amplification, sensitivity	Multiple steps, high background	[48,51]
Electrocatalytic label	Sensitivity (0.2 pM–400 pM)	Low-throughput	[52]
Quantum dots	Sensitivity (156 pM–20 nM)		[50]
T4 RNA ligase	Simple	ATP interference	[53]
Poly(A) polymerase	<3 fmol	Various limitations in labeling efficiency	[35]
Other technologies			
Northern blot	The most reliable technology – the 'Gold standard'	Radioactive isotopes, low throughput; low sensitivity	[23]
Cloning	Discovery of new miRNAs	High cost for sequencing	[24]
Real-time PCR	Rapid detection of miRNAs and precursors	High cost	[25,26]
In situ detection	Ability to detect miRNA levels in tissue	Low throughput	[54]
Bead-based	Multiple sample test, high speed and low cost	Specific conditions	[28]
Single molecule detection	Sensitivity (500 fmol)	High cost, special instrument	[55]

Abbreviations: Cy3, cyanine 3 (a fluorescent dye); RAKE, RNA-primed array-based Klenow extension.

ated the PureLink™ miRNA Isolation Kit, and Millipore (http://www.millipore.com) developed the Microcon® YM-100 for sRNA enrichment. In addition, sRNAs were isolated from total RNA using conventional denaturing polyacrylamide gel electrophoresis (PAGE) [43] and other procedures [44]. Thus, RNA molecules of ~200 nucleotides and less can be efficiently purified from the larger RNA species and sRNAs <40 nucleotides can also be highly enriched. These tools have proved invaluable for characterizing miRNA processing, facilitating the detection of miRNAs and avoiding the interference of signals from pre-miRNAs and other larger RNAs [21,34]. The techniques have been extended to the analysis of other important non-coding RNAs.

miRNA labeling and data analysis

The analysis of mature miRNA expression is key to understanding its physiological functions and pathological implications. In 2003, Krichevsky *et al.* [45] were the first to design an oligonucleotide array that could detect miRNAs in mammalian brain tissues by labeling low molecular weight RNA with radioactive isotopes. Other labeling technologies followed quickly (Table 1) [46–55]. In view of the extremely small size of miRNAs, direct labeling of miRNA molecules might be more advantageous. Even though there are several methods for direct labeling of miRNAs, direct enzymatic labeling of the mature miRNAs has often been used. In this reaction, T4 RNA ligase attaches one or two fluorophore-labeled nucleotides to the 3′ end of each miRNA. This labeling method leads to the highest activity without introducing bias. To minimize the interference of structure and sequence differences among miRNAs, Wang *et al.* introduced dimethylsulfoxide (DMSO), an effective RNA denaturant, into the reaction solution and found that up to 20% DMSO stimulated T4 RNA ligase activity [41]. It has been shown that this direct labeling method is an efficient tool for labeling miRNA and is easy to perform under laboratory conditions [34,41,54].

To obtain reliable results, microarray data must be background corrected and normalized before testing for significant differential expression and clustering and/or classification of genes that are potentially differentially expressed. Normalization is often performed to remove dye labeling bias and differences in hybridization and scanning. The spike-in controls included in different processing steps were used to remove miscellaneous variations so that biologically relevant alterations could be faithfully revealed [21]. It might be possible to use signals from a set of spiked-in synthetic sRNAs as common references. Once the normalization has taken place, the $\log_2$ ratios between sample and reference for each sRNA can be calculated, enabling the direct comparison of all $\log_2$ ratios from all arrays. Recently, Tang *et al.* introduced an additional array-data-adjustment step – northern blot analysis of a ratio of a given miRNA and U6 or tRNA – to validate results from ambiguous array data and to enable accurate data interpretation [56]. Moreover, it is important to use real-time PCR analysis for the validation of miRNA microarray data. Taken together, a set of standard methods for the normalization of miRNA data will be essential for effective analysis of miRNA expression.

Application of miRNA arrays

miRNA microarrays have been successfully used for the primary identification of new miRNAs that were predicted by bioinformatics approaches, for the dissection of differential expression of different miRNAs in the same cells, and for the comparison of miRNA expression profiles from different tissues or cells. These arrays have also been used for evaluating miRNA expression during development, differentiation, oncogenesis and other disease processes. In the following section we briefly describe some examples of successful applications of miRNA arrays in basic and clinical research.

Identification of novel sRNAs by arrays

Novel and previously unidentified miRNAs in animal and plant cells are often identified by northern blotting analysis or cloning technologies. These methods require a large amount of total RNA (hundreds of micrograms) as

starting material, and often also fail to detect miRNAs of low abundance. To overcome these obstacles, Grundhoff *et al.* [57] developed a new approach that combines computational prediction with subsequent microarray analysis. The relevant genomes were scanned computationally for miRNA precursors. New miRNAs predicted from this analysis were then included on the microarrays. Putative miRNAs identified in these arrays were then confirmed by northern blot analysis. Using this approach, the authors found a total of 18 new pre-miRNAs that give rise to 22 mature miRNA molecules. In a further development of this strategy, Berezikov *et al.* computationally predicted >800 novel mammalian miRNA candidates [58]. To verify these predicted miRNA candidate genes experimentally, the authors used the miRNA array approach that confirmed 348 novel mouse and 81 novel human miRNA candidate genes. They suggested that many miRNAs have been identified to date [58]. Our group has used bioinformatics to predict a novel class of sRNAs from human introns and we confirmed >200 sRNAs using a specialized sRNA array. [34]. Thus, the development of new bioinformatics approaches based on the structural features of different sRNAs, along with advances in experimental detection methods, such as sRNA microarrays, might help speed up the discovery of novel sRNAs from different species.

Tissue specificity of miRNAs

miRNA arrays can provide gene expression 'profiles' that represent detailed patterns of miRNA expression characteristic of cell or tissue responses to their microenvironment. It has been shown that different tissues express tissue-specific miRNAs, and furthermore that some miRNAs display different expression levels among different tissues. For instance, several studies found that miR-1, miR-133a and miR-206 were highly expressed in heart and skeletal muscle tissue compared with other tissues [59]. Ramkissoon *et al.* [60] reported that miRNAs such as miR-142, miR-155, miR-181 and miR-223 were expressed specifically in 17 malignant hematopoietic cell lines. Similarly, Sampere *et al.* identified a subset of brain-expressed miRNAs for which expression behavior was conserved in both mouse and human differentiating neurons [61]. It was recently indicated that miR-195 was upregulated during cardiac hypertrophy, but absent in the normal mouse heart [62]. Our results also demonstrated that different cells could share some common sRNAs and express their own unique sRNAs [34]. These results indicate that the patterns and levels of miRNA expression are different in diverse cells and tissues, suggesting that they might have important roles in maintaining tissue and cell identity or function, and that misregulation of miRNAs might lead to various diseases. In addition, these miRNAs could be used as biomarkers for discriminating between different cells and tissues.

Role of miRNA in stem cells

Stem cells have an important role in tissue generation, maintenance and repair. Recently, miRNA array technology has made significant contributions to the understanding of the regulation of stem cell functions through comparing sRNA expression profiling of different stem cell developmental stages. Evidence has emerged that miRNAs regulate the development of stem cells in plants and animals. Murchison *et al.* reported that on loss of Dicer, an essential enzyme in miRNA biogenesis, mouse embryonic stem (ES) cells showed division and proliferation defects, whereas re-expression of Dicer in the knockout mouse ES cells rescued these functions [63,64]. Houbaviy *et al.* identified 53 potential miRNAs in ES cells using a direct cloning approach and found that their expression was significantly reduced as ES cells differentiate into embryonic bodies [65]. Furthermore, a set of six clustered mouse miRNAs (miR-290–miR-295) was found to express specifically in ES cells. By contrast, Suh *et al.* demonstrated that miRNAs that were preferentially expressed in other tissues were poorly expressed, or not expressed at all, in ES cells [66].

Identifying miRNA expression patterns in stem cells has provided new insights into the understanding of stem cell renewal and differentiation, although these studies are in their infancy [34]. Successful combination of stem cells studies with miRNA analysis might yield extraordinary scientific and medical benefits. miRNA biology can be viewed as a crucial facet of stem cell function, particularly with regard to self-renewal and differentiation.

miRNA and cancer

Cancer is a genetic disease in most respects, including the presence of genetic alterations in both protein-coding genes and non-protein-coding genes. Recently, miRNA array data have contributed to the elucidation of the role of sRNAs in carcinogenic processes, such as mutagenicity, cell proliferation and cytotoxicity. miRNA arrays together with other molecular techniques, such as real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), have been used to identify and compare the miRNA expression profiles of normal cells and tissues with those in tumors such as lung cancer, colorectal neoplasia, glioblastoma, breast cancer, pituitary adenomas, thyroid cancers, lymphoma and other cancers [27,28,67,68]. These studies showed that distinct miRNA expression patterns were associated with various tumor types and that cancer samples had miRNA expression profiles that were clearly distinct from those of normal tissues. For instance, miR-126, miR-143 and miR-145 were expressed at significantly lower levels in >80% of the tumor samples compared with associated normal tissues, whereas miR-21 was found to be overexpressed in 80% of the tumor samples. The differentially expressed miRNAs could potentially represent two distinct pathways through which cancer suppressor genes and tumor genes could be suppressed and activated, respectively.

An array containing 217 miRNAs was used to evaluate tumors of unknown origin by Golub's laboratory [28]. These miRNAs were assayed on 17 poorly differentiated tumor samples with non-diagnostic histological appearance. The miRNA arrays could establish the correct diagnosis of the poorly differentiated samples with substantially greater accuracy (12 of 17 correct) compared with the mRNA-based classifier (1 of 17 correct), clearly demonstrating the advantage of miRNA profiles over mRNA profiles for diag-

nostic classification [28]. Recently, using miRNA microarrays, Bottoni *et al.* [69] found that 24 miRNAs represented a predictive signature of pituitary adenoma. These results suggest that miRNAs might regulate a wide array of growth and differentiation processes in human cancers and that the specific expression signatures of miRNAs could be a useful tool in classifying types of tumors and evaluating prognoses of cancers [29,30].

miRNA arrays have been used to identify new sRNAs and to analyze miRNA expression patterns in basic and clinical studies. It is already possible to link the growing body of information about miRNAs to high-throughput methods that assay differences in expression in different tissues and cells in both physiological and pathological stages. Integrating the data on sequence variation and expression variation will lead to the development of approaches that further risk assessment of patient subpopulations and establish more-specific diagnoses, therefore leading to selection of optimized therapies, as well as to improved monitoring of patients' responses to therapies. It is envisaged that these strategies could be used for a variety of diseases, particularly cancers.

Concluding remarks

Widespread and comprehensive use of miRNA microrrays will enable the identification of novel classes of sRNAs, quantification of miRNA expression and detection of miRNA expression patterns within and across species. Moreover, miRNA arrays can identify the expression of several hundred genes in the same sample at once while requiring only small amounts of total RNA. miRNA arrays will therefore increasingly contribute to both basic and applied research and promise to change the practice of medicine, potentially by providing the means for personalized diagnosis, cancer detection and prognostic assessment [67,68]. To fulfill the potential of miRNAs as a pivotal research and diagnostic tool, several challenges must be overcome.

The outcome of miRNA microarray studies depends on several factors of technical, instrumental and computational nature, and on materials, as well as on the interpretation of the raw data. For example, the poor storage of clinical samples, the quality of miRNA expression data and the comparison of different datasets require significant improvement. Indeed, a major limitation of miRNA microarray studies has been their lack of comparability and low accuracy of the derived data [30]. At present, no standard methodology exists for hybridization-based profiling of miRNAs and, as a consequence, comparison of expression data from different experiments can be difficult. To solve these practical problems it will be necessary to develop quality procedures for miRNA microarrays. Moreover, a set of commercially available standard miRNAs or samples would be helpful in comparing results from miRNA profiles for both basic research and clinical use. Thus, future miRNA microarrays could serve as platforms for the discovery of novel disease biomarkers for diagnosis of human disorders and for the identification and validation of therapeutic targets.

There are also needs for better understanding of the relatively new world of sRNAs. To date, the total number and types of sRNAs in humans remains to be determined. Their biogenesis and biological functions and modes of action in physiological and pathological states are still poorly explored and their regulatory mechanisms in cancers and other diseases remain unclear. Despite these limitations, the recent advances in the understanding of miRNA functions offer great promise that research findings can be translated into fully clinical application in the near future. Despite these outstanding challenges, we envisage that miRNA microarray platforms will prove to be important tools for producing high-quality, reliable data that will be useful in the discovery of novel sRNAs, in the understanding of miRNA functions, and in the diagnosis of various diseases.

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