miRNAs and their application in drug-induced liver injury

The complex miRNA regulatory network plays an important role in diverse biological activities of physiopathological processes. In addition to the discovery of and research on the extracellular miRNAs detected in multiple biofluids, the properties of tissue specificity and high stability underlie the great potential of these small miRNAs to serve as translational biomarkers for various diseases in the clinical setting, including in drug-induced liver injury. In this review, we describe the major technologies currently used and challenges in miRNA measurement and provide information on major bioinformatics resources available for current miRNA research. We also discuss novel findings in liver disease and highlight the potential of miRNAs for clinical and basic research as translational biomarkers for drug-induced liver injury.

KEYWORDS: drug-induced liver injury ■ microRNA ■ translational biomarkers

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Drug-induced liver injury (DILI) is a leading cause of acute liver failure in the USA and one of the key safety issues in drug development and postmarketing surveillance [1–3]. To date, the most commonly used biomarkers for DILI, such as ALT and AST, are not specific. There is a clear need for alternative biomarkers of DILI that possess more sensitivity and specificity for detection of DILI than the conventional markers. Identifying more reliable and specific biomarkers for DILI by using emerging and high-throughput technologies has been an active field of research. However, to date, the challenge remains not only the earlier identification of DILI and distinguishing severe DILI that can lead to life-threatening conditions, but also the accountability in translation to clinical use of such approaches. It is also important to discover translational biomarkers linked to mechanistic information about the injury using state-of-art technologies for drug development and clinical use.

Post-transcriptional regulation of genes is now recognized as an important factor of disease pathogenesis [4]. The discovery of miRNAs as a new class of regulators of gene expression has triggered an explosion of research activities and emerged as an important set of markers for drug discovery and development [5,6]. As key regulators in gene expression networks, miRNAs can influence different biological processes [7]. They have extremely interesting potential for disease-state biomarkers, and as well as in the toxicological context for tissue injury-specific biomarkers [8,9]. Unlike mRNA, miRNA has been shown to be remarkably stable in a variety of tissues and

body fluids [10,11]. This property greatly facilitates the use of miRNAs as translational clinical biomarkers of disease and injury [8,12,13]. The development of screening and diagnostic tests for various diseases based on analysis of miRNA in body fluids – mainly in plasma or serum, but also in urine, saliva, cerebrospinal fluid and milk – is a very active area of research [14]. Individual miRNAs and miRNA profiles have been applied as biomarkers for early diagnosis and prognosis in different clinical manifestations of liver diseases, including DILI [15–17].

In this review, we describe the strengths and pitfalls of the emerging methodologies and technologies utilized for miRNA analysis. We also provide a summary of information on the major bioinformatics resources available for the current miRNA research. Finally, we discuss miRNA as biomarkers in liver diseases and highlight the current understanding of the roles of different miRNAs and the application of miRNAs as potential translational biomarkers for DILI.

miRNA: biogenesis & function

miRNA are short (20–25 nucleotide) regulatory factors of gene expression [18]. Advanced studies have revealed unexpected diversity in their biogenesis pathways and regulatory mechanisms [7,19]. FIGURE 1 is a schematic diagram showing the process of miRNA biogenesis. Biogenesis of miRNAs is a multistep process [20] including the production of primary miRNA transcripts (pri-miRNA) transcribed by RNA polymerase II or III and cleavage of the primary miRNA to yield approximately 70–100 nt hairpin precursor miRNAs (pre-miRNA) by the microprocessor

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Figure 1. General mode of miRNA biogenesis.

RISC: RNA-induced silencing complex.

complex DGCR8 in the nucleus. The pre-miRNA is then exported from the nucleus by Exportin-5–Ran–GTP to the cytoplasm, then further processed by the RNase Dicer in complex to form a duplex of mature miRNA (miRNA/miRNA* duplex). Ultimately, the RNA duplex is unwound and the single-strand mature miRNA is incorporated into the protein complex to function as a guide, directing the silencing of target mRNA.

The control of miRNAs of gene expression is generally through inhibitory effects on protein translation by binding to the mRNA of their target genes [21]. Over the past decade, miRNAs have been shown to play important roles in biological systems and processes, such as organ development, cell proliferation and tumorigenesis [22–25]. Particularly, miRNA-based regulation has directly associated with disease etiology and treatment [26–30]. The discovery that a large amount of tissue-specific miRNAs exist in serum, plasma and other biofluids has led to intensive investigations on circulating miRNAs

and potential as biomarkers for diseases and drug development [17,31,32]. However, challenges still remain.

Technical challenges of miRNA isolation & detection

■ Current approaches & issues for miRNA samples preparation

miRNAs have been isolated from many body fluids, and most tissues [33–37]. Ideally, this isolation should be specific for miRNA. A more important approach is to elucidate tissue-specific miRNA functions by combining matching miRNA and protein expression data with miRNA target prediction and mechanistic models of gene network regulation. However, there are numerous technical variables that could affect miRNA analysis leading to erroneous results. This is a particular concern and critical when miRNAs are utilized as translational biomarkers in clinical application.

There are many products for miRNA extraction supplied commercially. Generally, these technologies designed for miRNA quantification

and integrity assessment use an Agilent Bio-analyzer [38–40]. Different extraction methods may influence the gene expression analysis of these small RNAs [40]. Major methodologies for miRNA extraction and quality considerations from various biological samples are summarized in TABLE 1. Generally, cultured cells and fresh frozen tissues produce high-quality miRNA that is suitable for miRNA expression profiling. However, most of biofluids are difficult specimens to work with due to the very low level of miRNA and other preanalytical variables. For example, miRNA detected in biofluids may have cellular pellets and an extracellular origin. However, only the latter is the most interesting in terms of biomarker discovery. Avoiding cellular contamination and hemolysis are therefore very important challenges [41,42]. Furthermore, biofluids, such as serum/plasma, contain inhibitors of the reverse transcriptase and polymerase enzymes used in the quantitative PCR (qPCR) reaction. In addition, miRNAs are secreted into blood circulation with extracellular vesicles, known as exosomes and microvesicles [43,44], or associated with Argonaute 2-containing protein complexes [45,46]. These extracellular vesicles play important roles in intercellular communication by serving as vehicles for transfer of cytosolic proteins, lipids and RNA between cell membranes. Thus, sample processing procedures that alter the vesicle contents may consequently influence miRNA profiles. Considering the relatively high cost and time consumption for miRNA expression profiling, more efforts should be put into the preanalytical variables to develop a standardized protocol for sample preparation and specific parameters for miRNA assessment.

■ Advantages & disadvantages of current technologies for miRNA expression profiling

miRNA analysis has provided great promise in basic and applied scientific research. Most

well-established molecular and biological technologies have been successfully transferred into miRNA research. A number of miRNA expression profiling methodologies have been developed, such as microarray and real-time reverse-transcription PCR (qRT-PCR) [47–49]. There is, however, a lack of consensus regarding optimal methodologies for miRNA quantification. The diverse methods for data analysis make the comparison of different platforms and integration of cross-platform miRNA profiling data even more challenging. A summary of the major platforms currently used in miRNA research is listed at TABLE 2. Each methodology has benefits and limitations. Over the last decade, microarray profiling and qPCR have provided great contributions to the evaluation of miRNA expression in the research community. However, limitations for these technologies still remain. As we know, miRNAs are significantly complicated compared with other nucleic acids owing to their biological properties and expression patterns. For instance, for the PCR technology, due to the very short length of mature miRNAs and the fact that many miRNAs differ by only one or a few bases, in general, miRNAs are not amenable for specific primer annealing under standard PCR conditions. Some unique signatures of miRNAs, such as their small total number (compare to other mRNAs), short length (20–25 nucleotides) and the low expression level for majority of miRNAs, have created technical obstacles for direct application in various array platforms. High-throughput, high-resolution next-generation sequencing (NGS) for miRNA profiling may offer promise to accurately identify the presence of known miRNAs and discover novel miRNAs in the near future.

Since its first use 8 years ago, NGS has been demonstrated as a potential platform for small RNA analysis and developed rapidly on its way to replace other technologies for DNA and

Table 1. Major biospecimens for miRNA extraction.

Sample type	General considerations on quality	Ref.
Cell lines	Usually high-quality miRNA	[99]
Cerebral spinal fluid	Can evaluate cell pellet and supernatant	[96]
Formalin-fixed paraffin-embedded tissue	More reliable than mRNA as an analyte in formalin-fixed paraffin-embedded tissue	[100]
Fresh frozen tissue	Cell type heterogeneity	[101]
Plasma or serum	RNases, low yield, generally, cannot measure quantity of extracted RNA	[102]
Urine	Can evaluate cell pellet versus supernatant	[103]

Table 2. Platforms comparison for miRNA profiling.

Items	Reverse-transcriptase qPCR	Microarray	RNA-seq
Principle	PCR application	Hybridization	Sequencing
Throughput	Medium to high	High	Ultrahigh
Total RNA required (ng)	500	100–10,000	250–10,000
Costs	Economic	Economic	Comparatively high
Dynamic range detected	Six orders of magnitude	Four orders of magnitude	Five or more orders of magnitude
Preferential field of application	Relative and absolute quantification; validation of other miRNA profiling approaches	Relative and absolute quantification of miRNA regulation; miRNA biomarker identification; routine application and higher throughput with respect to sample number compared with small RNA sequencing	<i>De novo</i> identification of small RNAs; simultaneous relative quantification of different small RNA species; holistic picture of the small RNA transcriptome
Advantages	Well-established method; sensitive and specific; can be used for absolute quantification of miRNAs	Well established; fairly low cost and high throughput	High accuracy in distinguishing miRNAs that is very similar in sequence, as well as isomiRs. Can detect novel miRNAs
Disadvantages	Only medium throughput with respect to the number of samples processed per day; cannot identify novel miRNAs	Lower specificity than reverse-transcriptase qPCR or RNA sequencing. Cannot identify novel miRNAs; difficult to use for absolute quantification	Substantial computational support needed for data analysis. Cannot be used for absolute quantification

qPCR: Quantitative PCR.

RNA analysis [50,51]. The increased resolution of NGS data provides a better understanding of the molecular processes that control the morphology and behavior of cells. NGS can provide unprecedented progress for insights into genomes [52]. Extensive studies using deep sequencing platforms have revealed unexpected complexity in relation to miRNAs, including 5'- and 3'-end-length heterogeneity and RNA editing. Moreover, the NGS systems directly count the number of transcripts found as a measure of expression abundance [53,54]. In particular, the technology is not limited by a predefined number of features, such as probe design, probe cross-hybridization or array background issues. Therefore, NGS is well suited to the discovery of novel miRNA species and small RNA transcription profiling, with an unprecedented sensitivity and resolution [55,56].

Although specific products and techniques vary, each platform is relatively stable in terms of its own miRNA profiling intrareproducibility; however, the poor interplatform consistency remains a great challenge for data integration and comparing across platforms. The best choice depends on the applications and a balance of cost, precision, accuracy and sample quantity. If the purpose is to screen a bunch

of samples to find a few altered miRNAs and you can tolerate certain false-negative results, the microarray may be the best platform. If the purpose is to detect individual miRNAs while the sample amount is a limiting step, qRT-PCR is the best choice owing to its sensitivity. If you are trying to find different isoforms or silent miRNAs and to discover novel miRNAs, then the NGS is the best approach.

Bioinformatics resources for miRNA target prediction & interpretation of discovered miRNAs

Controversially, despite advent in the development of technologies in detecting miRNA and more knowledge of the basic mechanism of action of miRNAs gaining, the main challenges for miRNA research still remain [57], for instance, the identification of miRNA targets to understand how they fine tune so many biological processes in both healthy and diseased tissue [58,59]. Toward this end, bioinformatics algorithms and computational approaches are playing a significant role in miRNA data analysis, including target identification [60]. The first computational miRNA target prediction approaches were developed independently by several groups during 2003 and

Table 3. Major bioinformatics resources for miRNA annotations.

Name	Development and maintenance	Content	Application
miRBase	www.mirbase.org	21,264 sequences of hairpin and 25,141 sequences of mature miRNA in 193 species, with many more new sequences are added regularly	A searchable database of published miRNA sequences and annotations for general purpose
miRecords	http://mirecords.biolead.org	miRecords consists of two components. The Validated Targets component hosts a large, high-quality manually curated database of experimentally validated miRNA–target interactions with systematic documentation of experimental support for each interaction. The Predicted Targets component of miRecords is an integration of predicted miRNA targets produced by 11 established miRNA target prediction programs. The current release of this database includes 1135 records of validated miRNA–target interactions between 301 miRNAs and 902 target genes in seven animal species	A resource for animal miRNA–target interactions and expected to serve as a useful for experimental miRNA researchers and informatics scientists developing the next-generation miRNA target prediction programs
DiANA TarBase	www.microrna.gr/tarbase	TarBase 6.0 hosts the largest collection of manually curated experimentally validated miRNA–gene interactions (more than 65,000 targets), presenting a 16.5–175-fold increase over other available manually curated databases	miTarBase can provide predicted regions by using a computational target prediction algorithm. The user interface provides links to external data sources such as NCBI Entrez, UCSC Genome Browser, miRBase, BioGPS, iHOP and HGNC. Optionally the user can submit data for nonindexed interactions
miRNAmap	http://mirnamap.mbc.nctu.edu.tw	Collection of experimentally verified miRNAs and experimental verified miRNA target genes in human, mouse, rat and other metazoan genomes	Three computational tools, miRanda, RNAhybrid and TargetScan, were applied for identifying miRNA targets in 3'-untranslated region of genes. The links to statistics related with miRNA sequences and target are very interesting
miR2Disease	www.mir2disease.org	A manually curated comprehensive resource of miRNA deregulation in various human diseases. The current version of miR2Disease documents 1939 curated relationships between 299 human miRNAs and 94 human diseases by reviewing more than 600 published papers. Each entry in miR2Disease contains detailed information on an miRNA–disease relationship, including miRNA ID, disease name, a brief description of the miRNA–disease relationship, miRNA expression pattern in the disease state, detection method for miRNA expression, experimentally verified miRNA target gene(s) and literature reference	miR2Disease provides a user-friendly interface for a convenient retrieval of each entry by miRNA ID, disease name or target gene
miRSEL	http://services.bio.ifi.lmu.de/mirsel	Documental database with associations between miRNAs and their targets using bibliographical searches, and offers the currently largest collection of literature derived miRNA–gene associations. miRSEL is updated daily and can be queried using a web-based interface via miRNA identifiers, gene and protein names, PubMed queries as well as gene ontology ('GO') terms	miRSEL combines text mining results with existing databases and computational predictions. Text mining enables the reliable extraction of miRNA, gene and protein occurrences as well as their relationships from texts

qRT-PCR: Real-time reverse-transcription PCR.

Table 3. Major bioinformatics resources for miRNA annotations (cont.).

Name	Development and maintenance	Content	Application
PhenomiR	http://mips.helmholtz-muenchen.de/phenomir	The content of PhenomiR is completely generated by manual curation of experienced annotators. Current version (2.0) released on November 2010 has 675 unique miRNAs mentioned in 362 articles that discuss 144 diseases and 98 bioprocesses	The web-based interface provides data from 542 studies that investigate deregulation of miRNA expression in diseases and biological processes as a systematic, manually curated resource
miRNA body map	www.mirnabodymap.org	The miRNA body map is a repository of qRT-PCR miRNA expression data and functional miRNA annotation in normal and diseased human tissues. The current version of the miRNA body map contains miRNA expression for 992 samples representing a total of 309,842 individual data points	The accompanying expression analysis pipeline allows analysis of miRNAs according to tissue specific expression, differential expression between tissues or sample subsets and functional annotation. The expression analysis pipeline is hierarchically structured and allows sample-centric, pathway-centric and miRNA-centric analysis in the available data sets
FAME	http://lacgt.cs.tau.ac.il/fame	FAME is a computational tool that uses predicted targets to automatically infer the biological process affected by a given human miRNA	A new permutation-based statistical method that tests for over- or under-representation of miRNA targets in a designated set of target genes

qRT-PCR: Real-time reverse-transcription PCR.

focus on prediction of the fruit fly and mammalian genomes [61–63]. With the progression of research on important roles of miRNAs in human diseases, several computational and experimental approaches toward miRNA target genes discovery have been developed and tested [64,65]. Major bioinformatic resources for miRNA research and their applications are listed in Table 3. Among these, miRBase is a comprehensive online repository of miRNA sequences and annotations. The most updated version of miRBase was released on 1 August 2012, containing 21,264 sequences of hairpin and 25,141 sequences of mature miRNA in 193 species (accessed date: 28 July 2013) and many more new sequences are added regularly [66]. In addition, as the dramatic increase of experimental data of miRNAs, more and more databases that curate and collect experimentally verified miRNA targets have emerged. For example, miRecords [67] and TarBase [68] provide computationally predicted or experimentally validated target genes for miRNAs and take into account the biological functioning of miRNAs, such as complex target gene-binding parameters.

Potential as translational biomarkers of miRNAs for DILI management

The miRNA profiling studies over the recent years have indicated the involvement of miRNAs in the pathobiology of various diseases including cancer [27,69], infectious diseases [70], diabetes, cardiovascular diseases, neurological diseases and immune system disorders [71–74]. The importance of miRNAs in liver disease is also being increasingly recognized [75,76]. Specific miRNA signatures have been identified in various etiologies of liver diseases, such as developmental disorders, fibrosis, hepatocellular carcinoma and hepatitis [77,78]. Summary of miRNAs in liver diseases are listed at Box 1. Among of them, miR-122 and 192, miR193 and miR155 have drawn great attention for their role in various liver diseases [79–81].

DILI represents a leading cause of acute liver failure, and major attrition to drug discovery and development [82,83], and has put the onus on pharmaceutical companies and regulatory agencies to protect the public health [84]. With the exception of acetaminophen, most drugs to cause DILI in humans in an unpredictable manner are cause idiosyncratic DILI [82,85]. Multiples factors have been reported to be associated with DILI occurrence, including genetic predisposition and environmental effects such as age, sex and obesity [86–88]. The difficulty in predicting DILI has prompted many studies to identify reliable and

specific biomarkers for liver injury. A number of exploratory biomarkers are currently under investigation [89,90]. miRNAs have been attracting great attention as potential biomarkers for liver diseases due to their tissue-specific nature and stability in biofluids. In addition, the high degree of cross-species conservation of miRNA sequences facilitates potential of translatability of miRNA biomarker assays from preclinical models and humans.

TABLE 4 lists a summary of major studies reporting miRNAs as potential biomarkers for DILI. To date, most available data on miRNAs in DILI are related to acetaminophen-induced acute liver failure. Indeed, and notably, many of the studies have demonstrated that levels of miR-122, a liver-enriched miRNA accounting for approximately 70% of total hepatic miRNAs, was increased many fold in the blood of DILI over the controls [17,91]. Expression of miR-122 is liver specific and inhibition of miR-122 expression in mice leads to a downregulation of cholesterol and lipid metabolism, specifically downregulating the key enzyme phosphomevalonate kinase. Remarkably, silencing miR-122 in high-fat diet-fed mice resulted in a significant reduction of hepatic steatosis, which was associated with reduced cholesterol synthesis rates and stimulation of hepatic fatty acid oxidation [92]. miR-122 targets the mRNAs encoding PPAR β/δ and SMARCD1/BAF60a, which are themselves circadian regulators of metabolism [93]. Another study shows that miR-122 controls systemic iron homeostasis in mice [94]. However, the mechanisms causing a decrease in levels of miR-122 after DILI are not clear. The most probable explanation is that damaged hepatocyte releases these miRNAs into the circulation. Current evidence suggests that serum miR-122 is at least as informative as ALT in both preclinical models and humans with acetaminophen-induced liver injury. To investigate the potential utility of miRNAs as markers of human DILI, Starkey Lewis *et al.* found that miR-122 correlated with peaks ALT, but not prothrombin time or total bilirubin concentration in acetaminophen patients [89,95]. Nevertheless, miR-122 alone may not be an ideal DILI biomarker because it does not distinguish benign clinical ALT elevations (e.g., following administration of heparin) from serious liver injury potential. In addition, miR-192 and miR-193 are reported to be down-regulated in a time- and dose-dependent manner in rats treated with acetaminophen [17,96]. Lack of understanding of the mechanisms underlying the roles of miR-122 and miR-192 in the biological

process of DILI leave many uncertainties about this miRNA as a translatable biomarker that can be utilized in the clinical practice.

Controversially, miR-155 has been reported to be associated with inflammatory liver disease and fibrosis. With an emerging role in regulating immune response, this miRNA is essential for normal B-cell differentiation and antibody production, and is involved in inflammation by regulating members of the superfamilies of TNF receptors and their ligands, such as TNF and TRAMP (TNFRSF25) [97]. In parallel with those identified genomic profiles, alcoholic liver disease (ALD) also correlated with deregulated levels of specific miRNAs. Recent research performed by Bala *et al.* provided important insights into the role of miRNAs in ALD [98]; the upregulation of miR-155, via NF- κ B signaling, might contribute to alcohol liver injury through

Box 1. Summary of deregulated miRNAs reported in liver diseases.

Liver development disorder

- ↓ miR-122
- ↓ miR-23b
- ↓ miR-30a/c

Fibrosis & cirrhosis

- ↓ miR-122
- ↓ miR-17-92
- ↓ miR-29
- ↓ miR-199a
- ↓ miR-221/222

Hepatocellular carcinoma

- ↓ miR-122
- ↓ miR-199a-3p
- ↓ miR-26a
- ↑ miR-155
- ↑ miR-181b/d
- ↑ miR-21
- ↑ miR-221/222
- ↑ miR-30d

Hepatitis

- ↑ miR-122
- ↓ miR-155
- ↑ miR-188a-3p
- ↑ miR-210
- ↑ miR-372-373

Metabolic disorder

- ↑ miR-103/107
- ↓ miR-122
- ↑ miR-33a/b
- ↑ miR-34a

Arrows indicate the alteration of the expression pattern of each miRNA in the designated disease.
↑: Upregulation; ↓: Downregulation.

Table 4. Reported studies on circulating miRNAs in drug-induced liver injury.

Liver injury	Species	Sample type	Method	miRNA expression trend	Ref.
DILI (APAP-induced)	Mouse	Plasma	SYBR® Green-based qPCR	miR-574-5p ↑, miR-135a* ↑, miR-466g ↑, miR-1196 ↑, miR-466f-3p ↑, miR-877 ↑, miR-342-3p ↓, miR-195 ↓, miR-375 ↓, miR-29c ↓, miR-148a, miR-652 ↓	[9]
APAP and CCl4	Rat	Serum and urine	SYBR Green-based qPCR	44 serum miRNAs and 28 urinary miRNAs ↑	[36]
ALD, DILI, induced inflammation	Mouse	Serum or plasma	TaqMan® qPCR	miR-122 ↑, miR-155 ↑, miR-146a ↑	[104]
Herb and APAP–DILI	Rat	Serum	TaqMan Rodent MicroRNA Low Density Arrays	miR-122 ↑, miR-192 ↑, miR-193 ↑	[8]
APAP–DILI	Human	Serum	TaqMan qPCR	miR-122 ↑, miR-192 ↑	[95]
Chronic hepatitis B, muscle disorder (human): DILI, ALD (mouse)	Human/mouse	Plasma	SYBR green-based qPCR	miR-122 ↑, miR-192 ↑	[105]
Hepatotoxic-induced injury	Rat	Plasma	TaqMan qPCR	miR-122 ↑	[106]
DILI	Mouse	Plasma	Mouse miRNA arrays	25 miRNAs ↑, 19 miRNAs ↓	[17]

‘**’ is considered as a minor product, which means miRNA-135a* has less concentration than miRNA-135a.

↑: Upregulated; ↓: Downregulated; ALD: Alcoholic liver disease; APAP: Paracetamol; DILI: Drug-induced liver injury; qPCR: Quantitative PCR.

activation of specific macrophages (Kupffer cells) by increasing TNF- α .

Opportunities & challenges in discovery miRNA as translational biomarkers for DILI in humans

The role of miRNAs as translational biomarkers for DILI is promising, particularly with regards to circulating miRNAs, which potentially offer a less invasive method of diagnosing liver injury. They have progressed from discovery to biomarker and remain at the forefront of research into disease pathogenesis and drug development programs. Despite significant advances in recent years, the miRNA mimic and antagonist technologies have not yet been validated as platforms for the development of safe and effective human therapeutics. In addition, prior to clinical application, there are major challenges such as the need for careful validation of diagnostic miRNA candidates in well-annotated clinical studies, as well as technical issues such as quantitation, standardization and normalization of expression. As a translatable biomarker, detection and quantification should be robust, rapid, simple, accurate, reproducible and inexpensive. miRNA detection currently falls short of all of these prerequisites. It is unlikely that any single miRNA will achieve the desired level of diagnostic or prognostic accuracy, as evidence is increasing that increased levels of particular miRNAs can be associated with several different types of diseases or functional changes.

The lack of association between miRNAs and insightful mechanistic information on DILI is another hurdle in the translation of miRNAs as biomarkers into the clinical setting. More work needs to be carried out to understand the complex biological function of miRNAs, their roles in pathogenesis and the safety of compounds that alter their levels. The extent and mechanism of function of extracellular miRNAs are largely unknown. It is believed that efforts to assess the utility of miRNAs for DILI as translational biomarkers can be better achieved by conducting a comprehensive investigation on the alterations of miRNAs in liver tissues treated by a large number of drugs that represent a broad range of DILI types and therapeutic uses. Such an exercise could provide more convincing evidence to elucidate the possible mechanisms underlying the phenotypes of DILI, and, potentially, translate the utilization of miRNAs biomarkers into clinical use. Active and vigorous investigations are ongoing in several laboratories across academia and industry to decipher the precise molecular functions and mechanisms of action for key miRNAs with potential for drug toxicity prediction. Such studies should lead to the development of reliable and cost-effective miRNA-based biomarker platforms for routine use in the DILI clinic in the next few years. Knowledge gained from these efforts will surely pave the way for designing safe drugs and will revolutionize the way we diagnose and predict toxicity including DILI.

Future perspective

In conclusion, there is considerable excitement surrounding the application of miRNAs in the biomarker field, which is well promised, given their stability and relative ease of detection. To bring these miRNAs into clinical use, cross-species validation, screening of additional hepatotoxicants and mechanistic-based information of corresponding miRNAs to DILI need to be investigated in future. We hope the advances in the analyses of circulating miRNAs reviewed here will lead to more efforts toward using miRNA as biomarkers of DILI, as well as other pathologies, and will facilitate the development of screening, prediction and monitoring tests for these diseases.

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Executive summary

Technical challenges in preanalytical processing of RNA samples

- miRNAs are a family of regulatory RNAs with a large role in multiple biology processes and functions.
- Lack of standardized protocols for miRNA isolation and quality assessment is a significant challenge for preanalytical processing for miRNA research.

Bioinformatics technologies for miRNA measurements & targets prediction

- Several computational and experimental approaches toward miRNA target gene discovery have been developed and are available for the research community.
- Identification of miRNA targets to understand how they fine tune so many biological processes in both healthy and diseased tissue needs deeper mechanistic study of miRNAs.

Potential of miRNAs as translational biomarkers for drug-induced liver injury management in humans

- miRNAs in liver disease: specific miRNA signatures have been identified in various etiologies of liver diseases.
- miRNAs in drug-induced liver injury (DILI): DILI can mimic most types of liver disease and share similar mechanisms. Thus, liver disease-specific miRNAs may be applicable for DILI.

Perspectives & challenges in discovering & translating miRNA biomarkers of DILI in clinical applications

- Promise: with regards to circulating miRNAs and liver specificity, miRNAs offer a great potential as novel biomarkers for DILI.
- Challenges: there is still need for more additional hepatotoxicants and mechanistic-based information on miRNAs in DILI as well as cross-species validation to facilitate the translation of miRNA biomarkers from preclinical setting to clinical application.

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