# Non-coding RNAs and drug-induced liver injury

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**Abstract:** Currently, more and more clinical cases of drug-induced liver injury (DILI) are increasing that may lead to acute liver failure and even death. It has been reported that DILI is the main cause of clinical drugs withdrawal, which results in a bad impact on the treatment of many diseases. So far there are still no effective monitoring means and therapeutic approaches for DILI. Nowadays many types of research on non-coding RNAs (ncRNAs), mainly including microRNAs, lncRNAs, and circRNAs, are interesting and have significant effects on lots of diseases, such as diagnostic biomarkers and treatment methods. This article mainly reviews concerning the ncRNAs in DILI to find the new sights of diagnosis and treatments in DILI.

**Keywords:** Drug-induced liver injury (DILI); non-coding RNAs (ncRNAs); microRNAs; exosomes

## Introduction

With the development and application of various drugs and the advanced progression of modern medicine, more and more clinical cases of drug-induced liver injury (DILI) are arising. It reported that the proportion of DILI is about 19 per 100,000 people (1,2). A multi-center, retrospective study recently reported that the incidence of DILI in mainland China is approximately 23.8 per 100,000 people. Moreover, the actual incidence may be higher since participants in this research are only composed of patients in hospital (3). A large prospective sample of studies based on the French population showed an annual incidence of 13.9 DILI per 100,000 inhabitants (4). Once adverse drug reactions occur, some symptoms will disappear or be alleviated if stop using them immediately. However, in some cases, it leads to acute liver failure that immediately require liver transplantation and even death. In the United States, DILI is the leading cause of acute liver failure, approximately accounting for about 50% (5). Moreover, DILI is also the main reason for drug withdrawal from pre-clinical research and markets (6).

Because the symptoms of DILI are similar to those of other liver diseases, there is no specific performance, which makes the clinical diagnosis more difficult. Data are showing that the incidence of DILI always changes, which signifies that there is still no simple and accurate methods for diagnosing DILI (7). Therefore, it is necessary to study the etiology and mechanism of DILI further and explore a new path for the diagnosis and treatments of DILI. The Human Genome Project shows that of the 3 billion base pairs that make up the human genome, only 1.5% of the nucleic acid sequences are used for protein-coding and the remaining 98.5% of the genomes are non-coding sequences (8). Besides the discrepancy between the increasing number of transcripts and a small number of known protein-coding genes can be explained by non-coding RNAs (ncRNAs) (9). ncRNAs have multiple biological functions, including regulating gene expression at transcription and posttranscriptional, which may affect chromosome structure, RNA processing and modification, mRNA stability and translation, and protein transport (10,11). They are widely involved in important life processes such as cell differentiation and

ontogeny, and their abnormal expression is closely related to various human diseases. ncRNAs are also known as a diagnostic and prognostic biomarker (12,13). They also perform broad application prospects as biomarkers in the diagnosis and prognosis of DILI. Here, we will review the relationship between ncRNAs and DILI and provide ideas for the diagnosis and treatments of DILI.

## Risk factor, current diagnostic tools for DILI

The liver is an important organ for drug metabolism, which becomes the basis for the formation of DILI. The drug is metabolized in the liver and involves three steps, the first involves cytochrome P450, the second phase involves glutathione transferase and n-acetyltransferase, and the third step involves transporting the protein to form a compound for transport excretion. The liver converts it into a water-soluble metabolite and excretes it. Cytochrome P450 eliminates smaller parts by N-hydroxylation, forming the metabolite N-acetyl-p-benzoquinone imine (NAPQI), a metabolite that damages cells. A large proportion of acetaminophen (APAP) is biologically activated to NAPQI, which may deplete glutathione storage in the liver. When glutathione depletion reaches a critical level, NAPQI reacts with cellular structure and causes hepatocyte damage (14). Risk factors for DILI include drug dosage, drug lipophilicity, and liver metabolism. The study showed that compounds with high levels of liver metabolism had a three-fold increase in alanine aminotransferase (ALT) levels and are easy to develop liver failure, liver transplantation, and death. The study also found that hepatotoxicity was associated with compound dose, with significant liver metabolism and daily dose. When the dosage surpassed 50 mg of the compound has significantly higher hepatotoxicity (15). There is evidence to support the role of host factors such as age, gender, and chronic liver disease in the development of DILI. For specific drugs, genetic susceptibility appears to be a risk factor for DILI (16).

DILI is divided into intrinsic and specific liver injury. The former is related to the dose of the drug, which can be predicted. The latter is various and unpredictable (17). Therefore, most studies are focused on specific drug-induced liver damage. There are many drugs with hepatotoxicity including tyrosine kinase inhibitors, monoclonal antibodies, new oral anticoagulant drugs, new antiplatelet drugs, antibiotics, antidiabetic drugs, antiepileptic drugs, antidepressants, antipsychotics, and retroviral drugs (18). Anti-tuberculosis drugs are the most

common cause of specific drug-induced liver damage worldwide (19). Hepatic injury induced by APAP in the United States is a common cause of acute liver failure (20,21). The latest survey shows that Chinese medicine, Chinese herbal medicines, and dietary supplements, as well as anti-tuberculosis drugs, are the main causes of drug-induced liver damage in mainland China (3).

There is currently no specific diagnostic tool for DILI. Biomarkers or methods for assessing DILI currently include biochemical markers such as aminotransferases, total bile acids, histopathology or ultrastructural pathology, active metabolites, and immunologically relevant markers. Although ALT is a routine test for the diagnosis of liver disease, patients with burns, muscle inflammation and with hypothyroidism have also elevated levels of extrahepatic ALT (22). Moreover, it usually takes 72 hours for the ALT level to peak. Nowadays there are also emerging biomarkers that can be evaluated for DILI including total keratin 18 (K18) and caspase-cleaved K18 (ccK18), macrophage colony-stimulating factor receptor 1, high mobility family box 1 and miR-122 (17). The FDA defines the characteristics of Hy's law to identify the potential DILI patient: first, ALT or AST in patients with DILI is more than three times higher than the upper limit of normal. Second, the first condition is met, and total serum bilirubin is more than twice the upper limit of normal. No cholestasis disease was found.

Moreover, it is impossible to explain the simultaneous increase of transaminase and serum total bilirubin in the condition of other diseases. Ten percent of patients who meet Hy's law will develop a severe DILI, which requires liver transplantation or even death (17). Liver biopsy is an important tool for the diagnosis of DILI, providing clues to potential pathogenesis, providing prognostic information and guiding treatment (23). However, the liver puncture is an invasive examination. In clinical practice, limitations due to patient's willingness and operational contraindications, due to the limitation of patients' willingness and operation contraindications, the implementation rate of liver biopsy is far from meeting the needs of clinical diagnosis. The FDA also uses Hy's law as a predictor of severe toxicity in clinical trials, and other strategies at the clinical include diagnostic biomarkers and genetic polymorphism assessments that may predict DILI susceptibility (7). Cases of suspected specific DILI should be classified as hepatitis, cholestasis or mixed according to the degree/proportion of ALT and alkaline phosphatase abnormalities. The cause of other liver diseases should be carefully evaluated, but a liver biopsy is

rarely needed (24). Antioxidants are also considered to be a treatment for severe DILI, while N-acetylcysteine (NAC) is the first choice for the treatment of APAP overdose (7,25).

## ncRNAs in DILI

Non-coding RNA accounts for the vast majority of the human genome, whose functions are largely unknown (26). Modern high-throughput sequencing analysis identified a large number of non-coding ncRNAs, including miRNA, siRNA, piRNA, snRNA, snoRNA, circRNA, lncRNA and so on. They play an important role in physiological and pathological conditions, and current researches indicate that ncRNAs can be used as diagnostic markers and therapeutic targets (27).

### MiRNAs in DILI

#### Biogenesis and functions of miRNAs

The microRNAs (miRNA, miR) are single-stranded ncRNAs consisting of approximately 21 nucleotides. MicroRNAs first synthesize pri-miRNAs containing a stem-loop structure by RNA polymerase II, cut by the combined effects under RNase III Drosha and cofactor complex subunit DGCR8 in the nucleus, and form approximately 65 bases in length called pre-miRNAs (28,29). Then the pre-miRNAs are transported into the cytoplasm by export protein 5. This process that transporting pre-miRNAs requires to obtain energy by hydrolyzing GTP. The pre-miRNAs released into the cytoplasm is further processed by Dicer into miRNAs of approximately 21 nucleotides in length, which is then further processed by argonaute protein (AGO) to form mature miRNAs (30,31). It is capable of inhibiting the translation of mRNA by targeting the 3'-UTR of the target mRNA. Also, when the miRNAs perfectly match with the 3'-UTR of the target mRNAs, RNA induced silencing complex (RISC) form and then enter the cytoplasm to degrade the target mRNA to regulate gene expression (32). In addition to inhibiting targeted mRNA translation, miRNAs can also activate translation through non-canonical pathways in the latest research findings. For example, miR-328 competes with CEBPA mRNA for binding to hnRNP E2 translational regulator poly-binding protein, while hnRNP E2, in turn, releases CEBPA which can be translated to proteins (33). Human miR-369 directs the binding of AGO2 and fragile X mental retardation-associated protein 1 and AU-rich elements (ARE) in TNF-α mRNA to activate translation (34). The researches

have been explored to investigate the functions of miRNAs in many clinical diseases. Here we mainly summarize the significance of miRNAs in DILI.

#### MiRNAs in the pathogenesis of DILI

There are several reasons for the changes of miRNAs in DILI: firstly, microRNAs are transferred to other compartments through exosomes, where they enter cells horizontally and act as signaling molecules. Secondly, miRNAs may leak or release from cells due to membrane damage and necrotic cell death.

Little is known about the role of individual miRNAs and their targets in immune and inflammation-related responses in DILI. In a certain study, a mouse model of liver injury induced by methimazole was used to study the involvement of miRNAs in T helper type 2 immune responses (35). MiR-155 is a multifunctional microRNA that is known to regulate inflammatory responses by regulating various target genes. The study showed that miR-155 protects against APAP-induced liver injury by mediating NF-κB signaling (36). This suggests that miR-155 may be a potential therapeutic target for APAP-induced hepatic inflammation (36).

APAP overdose is the major cause of acute liver failure worldwide, in which mitochondrial DNA released by damaged hepatocytes activates neutrophils through the binding of TLR9 and further aggravate liver damage (37). Here, the authors demonstrate that mtDNA/TLR9 activates a negative feedback pathway to limit neutrophil overactivation and liver damage by miR-223. Activation of TLR9 up-regulates miR-223 by enhancing NF-κB binding on the miR-223 promoter, whereas miR-223 attenuates TLR9/NF-κB-mediated inflammation by targeting IKKα expression. In general, upregulation of miR-223 plays a key role in terminating acute neutrophil response and is a therapeutic target for the treatment of APAP-induced liver failure (37).

Integrated microRNA-mRNA methods studied the dose- and time-dependent effects of triterpenoid toosendanin (TSN) on mouse liver. Pathway analysis based on the intersection between microRNAs differentially expressed at three-time points and predicted targets of differentially expressed mRNA reveals that TSN-induced liver injury may be caused by glutathione depletion, mitochondrial dysfunction, and lipid metabolism disorders, ultimately leading to the liver Hepatocyte necrosis. The research suggested that integrated microRNA-mRNA methods can provide new insights into the complex and dynamic

behavior of TSN-induced liver injury (38).

The APAP is the most common cause of DILI in the UK and USA (39). The study discovered that APAP overdose for 24 hours resulted in more than two-fold changes in plasma miRNAs with elevations of 25 miRNAs, and the most significant increase is miR-122 (22). The study also indicated that the highest expression of miRNAs in the liver was miR-711 and the largest decrease in miRNAs was miR-29b (22). Changes in the spectrum and levels of miRNAs in the liver and plasma in the toxin exposure-response are different. The APAP overdose-induced a significant change in the expression level of miRNAs in liver tissues. Comparing the damaged tissue with the control group, 18 miRNA levels were decreased, and 33 miRNA levels were elevated. However, only 20 of these altered miRNAs have changed in plasma (22).

Advances in technology have greatly promoted the development in the field of biomarker research, especially when high-throughput sequencing emerges. A total of 33 miRNAs and three novel miRNA-like snRNAs were identified by next-generation high-throughput sequencing in acetaminophen-administered patients (40). miR-22, miR-27b, and miR-30a were previously found to be enriched in plasma of acetaminophen-overdosed mice (40). Hundreds of miRNAs that were significantly elevated in the serum of patients with APAP excess were identified by quantitative real-time PCR. Most of these circulating microRNAs can be reduced to normal levels during treatment with NAC. Although most elevated circulating miRNAs are restored under the successful NAC treatment, miR-1290 is still elevated for at least two days after other miRNAs begin to decrease to normal levels (41).

Of the 528 murine miRNAs analyzed, more than 40 potential miRNAs were up-regulated and down-regulated by a lethal dose (500 mg/kg) more than 2-fold compared to the APAP sublethal dose (150 mg/kg), especially miR-574-5p, miR-135a, miR-466g, miR-1196, miR-466f-3p and miR-877, were up-regulated in a lethal environment compared to sublethal APAP-associated hepatotoxicity, while miR-342-3p, miR-195, miR-375, miR-29c, miR-148a, and miR-652 were significantly down-regulated (42). Interestingly, we found that the highest 12 folds of the top 12 miRNAs related to asthma compared to the sublethal dose of APAP mice (42). The whole blood miRNA concentrations increased 4 h after acute liver failure and were associated with molecules associated with injury in porcine models of acetaminophen-induced acute liver failure (43). Plasma miR-122 levels began to increase before ALF and continue

at a high level until the patient dies, while plasma miR-192 levels increased from ALF development and increased from 8 hours after ALF to death. By quantifying individual tissue-specific miRNA species, we were able to visualize the timeline of organ damage in the model, starting with the liver at the ALF point, then the kidney, and finally the end of the brain (43).

There is a study identified potential biological pathways associated with monocrotaline-induced liver injury in mice through integration analysis of liver microRNAs and mRNA. The results of mRNA chip analysis showed that the expression levels of 569 genes were up-regulated, while the other 417 genes were down-regulated in MCT-treated mice (44). The miRNA microarray analysis showed that the expression level of 15 miRNAs was significantly altered in the liver of MCT-treated mice and 11 of these miRNAs were validated using Real-time PCR assay which has 426 target genes (44).

### MiRNAs as biomarkers and therapeutic tools in DILI

#### microRNAs in body fluids

The discovery of circulating miRNAs in urine and other body fluids has provided a new way to detect non-invasive biomarkers of organ damage. For example, urine-derived miRNAs may be useful biomarkers for kidney and bladder diseases, even DILI. Urine miRNAs appear to be another form of non-invasive DILI biomarkers and can be used for the classification of hepatotoxins. MiR-122 is one of the most abundant miRNAs in liver tissue and is tissue-specific, accounting for approximately 72% of total liver miRNAs (45). Increased miR-122 was found in the plasma of patients and animal models of viral, alcoholic, and chemically induced liver disease and this change were associated with liver histological changes (46). The researchers used a systematic review of the literature and conducted a meta-analysis to conclude that miR-122 has high sensitivity and specificity in the diagnosis of DILI (47). The study further indicated that the specific circulating microRNAs such as miR-122 and miR-192 might be more sensitive biomarkers to detect acetaminophen-induced liver damage compared to ALT (47). It has been demonstrated in rats (48), humans (49), and mice (22) that miR-122 is an APAP-DILI biomarker that is easier to detect than ALT. Inhibition of miR-106b expression and subsequent upregulation of STAT3 is critical for the pathogenesis of HAL-induced liver injury (50). At 24 hours after the administration of trovafloxacin, miR-877-5p is the most abundant miRNA in the liver of mice, and increased

miR-877-5p-induced PEPCK may be a trigger for the development of trovafloxacin-induced liver injury (51). miR-122-5p, let-7c-5p, and miR-148a-3p promote a mammalian liver-specific phenotype, and miR-92a-3p, cell proliferation, and angiogenesis promoter, plasma miR-122-5p can be considered as a powerful new generation of fish biomarkers, miR-122-5p as a plasma biomarker of liver injury in fish exposed to microcystin-LR (52). A group of 11 miRNAs was identified, and their distribution and dynamics in circulation during NAC treatment can distinguish between APAP liver damage and ischemic hepatitis (41). These miRNA-based markers represent a potentially more sensitive and reliable assay for drug-induced tissue damage.

Moreover, miR-122 is a potential use as a human DILI biomarker when measured in capillary blood drops, which can be used as a minimally invasive technique to detect patients suspected of DILI more simply and conveniently (53). A single probe for the detection of human serum microRNAs using a single molecule array with sequence specificity up to a single base without polymerase amplification and the serum miR-122 levels are higher in all liver injury patients and measured. This method allows rapid quantification of circulating miR-122 (54).

## microRNAs in exosomes

Exosomes are membranous vesicles with a diameter of 30–100 nm that are released from the multivesicular body of eukaryotic cells and released into the cell membranes (55). The exosomes are spherical, cup-shaped or flat-shaped. They are mainly formed by intracellular lysosomal microparticles, which are released into the extracellular matrix after fusion with the cell membrane through the outer membrane of the multivehicle. Almost all exosomes are rich in four transmembrane protein families (such as CD63, CD81, CD82, CD53, etc.), which play an important role in cell invasion and fusion (56). Exosomes are cell-derived vesicles found in many biological fluids, including urine, blood, ascites, and cerebrospinal fluid (57), which contain biologically active proteins, lipids, mRNAs, and other ncRNAs. The substance is easier to fuse with the cell membrane of adjacent cells, and selectively delivers biologically active substances to the recipient cells, transmits information between different cells, regulates signal transduction between cells, and exerts various biological functions.

Studies have shown that microRNAs in plasma and plasma-derived exosomes are differentially regulated under certain disease conditions (58). Exosome miR-122a-5p has an earlier diagnostic potential and more diagnostic time in acetaminophen-induced liver injury than in serum, and studies have shown exosome microRNAs, and ALT levels in APAP-induced DILI have a higher correlation (59). Moreover, exosomal microRNAs are more stable than non-vesicular microRNAs in plasma (60). These results strongly suggest that exosome microRNAs are expected to be better and more stable than plasma microRNAs as biomarkers for the detection of DILI. In addition to APAP-induced DILI, Chinese medicine is also a large class of drugs that cause DILI. Studies have shown that compared with the control group, Chinese medicine such as Chuanxiong Zi has differential expression of exosome microRNAs in liver injury, including serum exosomal miR-370-3p, which is the most significant microRNA downregulated (61). This series of findings allow us to understand the exosomes involved in DILI, but there should still be many unknown exosomal mRNAs and microRNAs involved in DILI, which is yet to be explored.

Urine collection is non-invasive and can be collected in large quantities as a unique advantage of urine exosomes as a DILI biomarker. Urine extracellular vesicles have been shown to carry different types of kidney disease biomarkers (62). The miRNAs secreted by cells are present in the extracellular environment, including urine, in a complex of microvesicles, exosomes, and proteins. Currently found in a rat model of APAP or carbon tetrachloride-induced liver injury, Increased urine miRNA (63). A recent study analyzed the levels of exogenous protein in the urine of DILI rats, indicating that PrPc, Cd26, Slc3a1, Cd81, and Cd10 in urine microvesicles can be used as candidate biomarkers to help diagnose liver damage (64).

## *Other ncRNAs and DILI*

LncRNA is also a member of ncRNA, which refers to an RNA transcript of more than 200 nucleotides in length and which does not encode a protein. It is located in the nucleus or cytoplasm and is mainly transcribed from RNA polymerase II and can be selectively polyadenylated or spliced. It has a promoter structure and is widely expressed in the genome. Play an important role in transcriptional regulation, post-transcriptional regulation, protein metabolism and chromatin remodeling (65,66). lncRNA can regulate gene transcription through a variety of mechanisms, both by regulating the local genes of transcription itself and by binding to protein complexes to regulate gene expression. At the same time, lncRNA

can also form miRNAs to form RNA degradation complexes and degrade miRNAs. lncRNA can also directly regulate its transcription with mRNA, regulating final protein expression and terminal differentiation (67,68). CircRNA is a special non-coding RNA discovered in recent years. Unlike linear RNA, there is no 5' "hat" and 3' polyadenylation "tail", which is a closed loop structure, through lasso or back-splicing, a special end-to-end annular structure is formed. The circRNA structure is stable, evolutionarily conserved, and has significant expression specificity that confers different functions to the circRNA. Recent research has revealed that circRNAs can function as miRNA sponges, regulators of splicing and transcription, and modifiers of parental gene expression (69). There is a view that circRNA can be used as a molecular marker for complex diseases (70). There have been many studies on these RNAs and other diseases, but it has not yet explored the relationship with drug-induced liver damage, which is a pity. Similar to miRNAs, ncRNAs also have important regulatory effects on gene transcription and translation. In the future, we can explore the relationship between other ncRNAs and DILI.

## Conclusions and prospect

Considering that the pathogenesis of DILI is particularly complex, the main process of DILI occurrence is still unclear. It has been found that drug-induced liver damage affects a variety of microRNAs, but the targets of many microRNAs are not understood completely, and there are some differences between certain microRNA targets, which are obstacles to DILI research. miRNA can be used not only for early detection of DILI but also for distinguishing between different types of DILI. More research is done on miRNAs that are specific or highly expressed in a particular tissue, as this can provide insight into the functional role of miRNAs in various tissues. In this regard, further functional studies of exosome miRNAs as signaling molecules between different cells and organs can contribute to early detection of liver injury as well as stratification of patients based on the severity and type of liver injury. Given the therapeutic and diagnostic potential of miRNAs, the main challenge is to integrate these findings into the clinical setting that can be used to treat DILI cases. Also, however, gradually overcoming these hurdles, continuing to study DILI, and providing a detailed explanation of the role of microRNAs in the development of DILI can provide more clinical diagnosis, therapeutic use, and prognostic evaluation. Other

ncRNAs include lncRNAs, which play an important role in a variety of diseases but are currently underrepresented in DILI. With the rapid development of biotechnology and increasing awareness of these ncRNAs, these ncRNAs may affect normal gene expression and disease progression, making it a new class of targets for drug discovery (26,71). ncRNA is a novel and challenging potential drug target. Many potential problems may have been addressed in the development of technologies that target mRNA, and this view will first consider the lessons learned from nucleic acid-based targeted mRNAs including those related to targeting specificity and toxicity. Drug discovery applied to ncRNA also can learn from this experience.

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## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/ncri.2019.07.01). The authors have no conflicts of interest to declare.

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