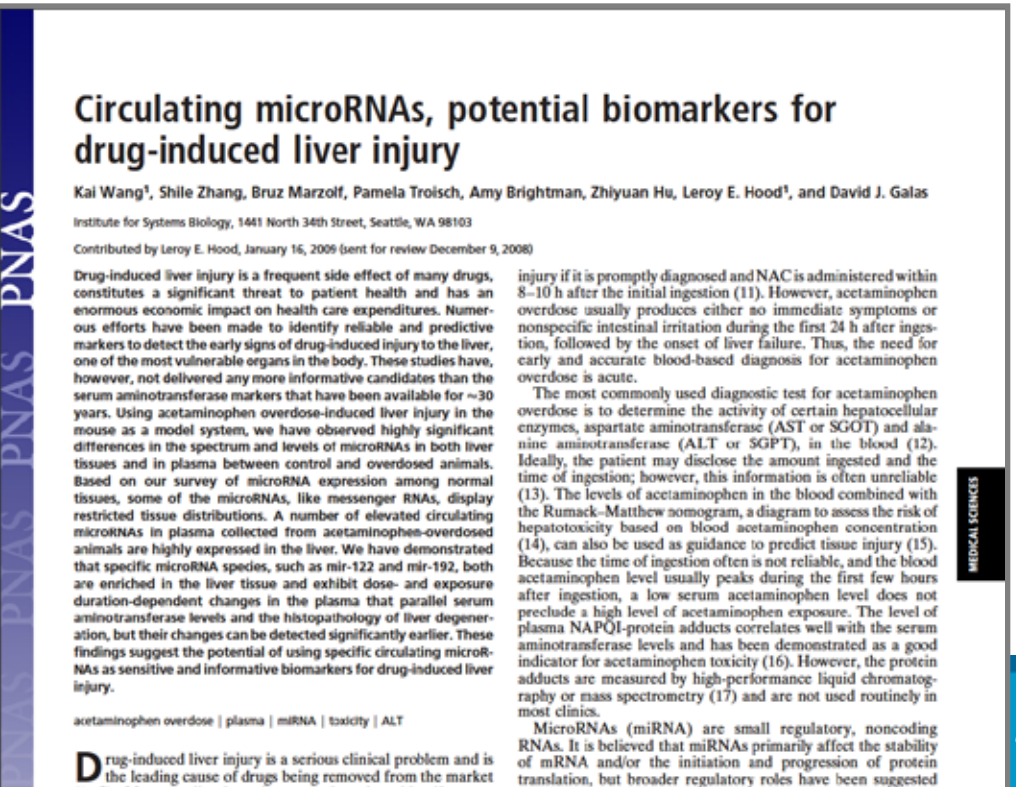
Circulating microRNAs, potential biomarkers for drug-induced liver injury

Microarray-based analysis of microRNA expression levels reveals candidates for early markers of disease

A literature Review

Wang et al., PNAS 2009



PNAS

MEDICAL SCIENCES

Circulating microRNAs, potential biomarkers for drug-induced liver injury

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Contributed by Leroy E. Hood, January 16, 2009 (sent for review December 9, 2008)

Drug-induced liver injury is a frequent side effect of many drugs, constitutes a significant threat to patient health and has an enormous economic impact on health care expenditures. Numerous efforts have been made to identify reliable and predictive markers to detect the early signs of drug-induced injury to the liver, one of the most vulnerable organs in the body. These studies have, however, not delivered any more informative candidates than the serum aminotransferase markers that have been available for ~30 years. Using acetaminophen overdose-induced liver injury in the mouse as a model system, we have observed highly significant differences in the spectrum and levels of microRNAs in both liver tissues and in plasma between control and overdosed animals. Based on our survey of microRNA expression among normal tissues, some of the microRNAs, like messenger RNAs, display restricted tissue distributions. A number of elevated circulating microRNAs in plasma collected from acetaminophen-overdosed animals are highly expressed in the liver. We have demonstrated that specific microRNA species, such as mir-122 and mir-192, both are enriched in the liver tissue and exhibit dose- and exposure duration-dependent changes in the plasma that parallel serum aminotransferase levels and the histopathology of liver degeneration, but their changes can be detected significantly earlier. These findings suggest the potential of using specific circulating microRNAs as sensitive and informative biomarkers for drug-induced liver injury.

acetaminophen overdose | plasma | miRNA | toxicity | ALT

Drug-induced liver injury is a serious clinical problem and is the leading cause of drugs being removed from the market

injury if it is promptly diagnosed and NAC is administered within 8–10 h after the initial ingestion (11). However, acetaminophen overdose usually produces either no immediate symptoms or nonspecific intestinal irritation during the first 24 h after ingestion, followed by the onset of liver failure. Thus, the need for early and accurate blood-based diagnosis for acetaminophen overdose is acute.

The most commonly used diagnostic test for acetaminophen overdose is to determine the activity of certain hepatocellular enzymes, aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT), in the blood (12). Ideally, the patient may disclose the amount ingested and the time of ingestion; however, this information is often unreliable (13). The levels of acetaminophen in the blood combined with the Rumack–Matthew nomogram, a diagram to assess the risk of hepatotoxicity based on blood acetaminophen concentration (14), can also be used as guidance to predict tissue injury (15). Because the time of ingestion often is not reliable, and the blood acetaminophen level usually peaks during the first few hours after ingestion, a low serum acetaminophen level does not preclude a high level of acetaminophen exposure. The level of plasma NAPQI-protein adducts correlates well with the serum aminotransferase levels and has been demonstrated as a good indicator for acetaminophen toxicity (16). However, the protein adducts are measured by high-performance liquid chromatography or mass spectrometry (17) and are not used routinely in most clinics.

MicroRNAs (miRNA) are small regulatory, noncoding RNAs. It is believed that miRNAs primarily affect the stability of mRNA and/or the initiation and progression of protein translation, but broader regulatory roles have been suggested

Agilent Technologies

Plasma MicroRNA as Predictive Markers

Goal:

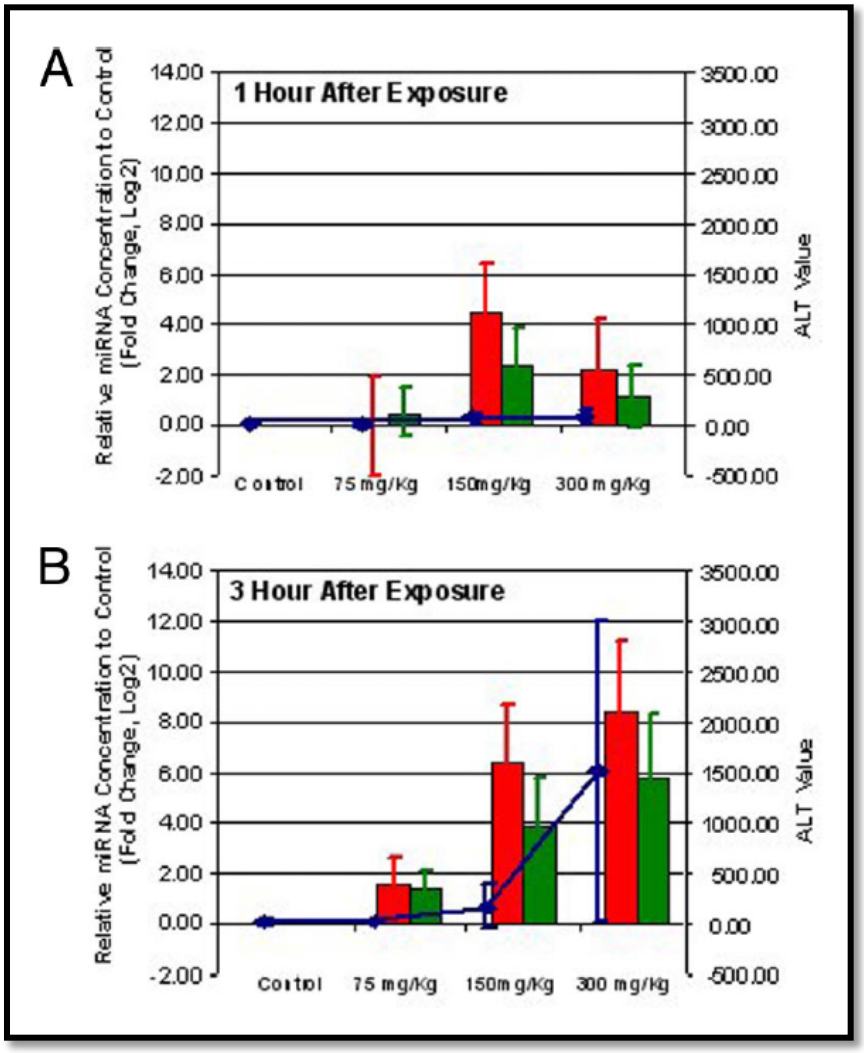
- Identify early signs of acetaminophen-induced injury to the liver

Approach:

- Compare mice with and without overdose-inducing drug treatment
- Profile microRNAs in plasma and liver using the Agilent miRNA microarray and Agilent labeling, hyb, and wash kits
- Find microRNAs that correlate to dose, exposure, and liver injury
- Confirm predicted expression changes using SYBR green-based qPCR

Wang et al., PNAS 2009

Circulating miRNAs as Early Predictors of Liver Injury



Drug exposure response:

- **ALT**, a commonly-used diagnostic indicator for acetaminophen overdose, did not respond to 1 hour treatment
- **mir-122** and **mir-192** had elevated expression levels after only one hour
- After 3 hours, **ALT**, **mir-122**, and **mir-192** all showed responses to drug treatment proportional to dosage

Drug dosage response:

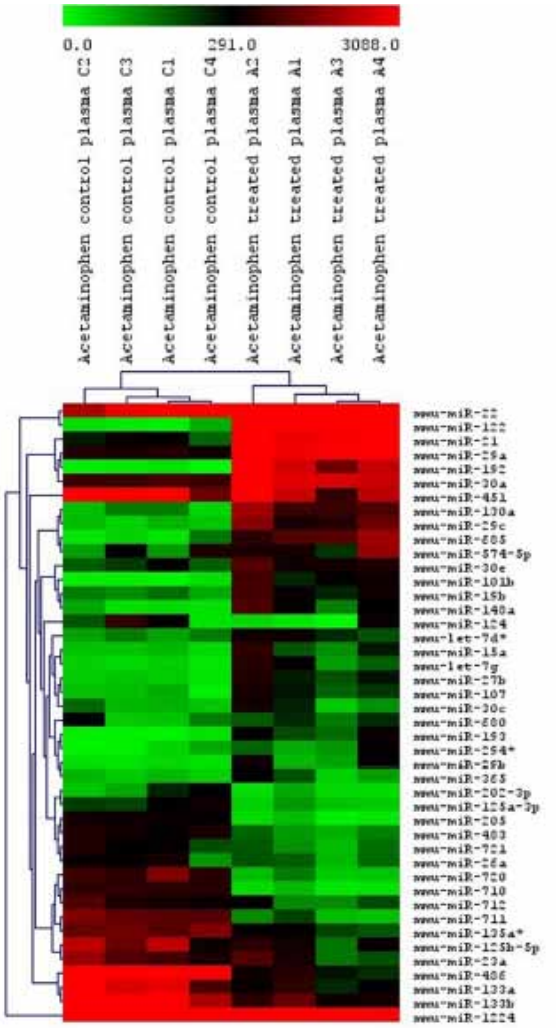
- **mir-122** and **mir-192** dosage sensitivity began at 75 mg/kg
- **ALT** sensitivity began at 150 mg/kg

Ø **Circulating miRNAs showed earlier and more sensitive responses to dosage and exposure than a commonly used diagnostic marker**

Wang et al., PNAS 2009

Features of Plasma and Liver microRNA Expression

- Correlation of miRNA expression among biological replicates:
  - *Without drug*: r = 0.95 to 1.00 (N=4)
  - *With drug*: r = 0.73–0.99 (N=4), (suggesting some variation in drug response)
- Dynamic range of miRNA expression levels in both plasma and liver:
  - 4 orders of magnitude
  - Consistent with various tissues and cell types



44 miRNAs showing a 2x change between control and drugged plasma samples.

Wang et al., PNAS 2009

In Summary

- Profiles of circulating microRNAs in the plasma, based on Agilent microarray analysis, revealed novel biomarkers predicting drug-induced liver injury
- Selected microRNAs appeared more effective as biomarkers than a commonly used diagnostic marker:
  - Earlier response to drug
  - Increase sensitivity to drug dosage

The authors state:

- *Our results, although preliminary, strongly suggest that a more comprehensive study of circulating miRNAs and their association with various physiopathological conditions may lead to another dimension in the discovery of biomarkers in the blood for many physiological and pathological conditions, including toxicity.*

Open Genomics

Wang et al., PNAS 2009