Serum microRNA biomarker identification in a residential cohort with elevated polychlorinated biphenyl exposures

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Introduction

Toxicant-associated steatohepatitis (TASH) is a form of liver disease associated with both industrial [1] and environmental [2] chemical exposures. Like other forms of Non-alcoholic Fatty Liver Disease (NAFLD), TASH can contribute to systemic metabolic disease states and may progress to cirrhosis and increase risk for cancer. Unlike other forms of NAFLD, however, TASH is unlikely to be detected incidentally or through conventional screening tests such as serum alanine transaminase [ALT] or aspartate transaminase [AST] activity [1].

New liver injury biomarkers that can detect this unique form of steatohepatitis will help identify subpopulations susceptible to advanced liver diseases and associated metabolic conditions. MicroRNAs (miRs) are non-coding regulators of gene transcription and translation that maintain cellular homeostasis and mediate responses to environmental exposures. In recent years, miRs have been found to be stable in accessible matrices such blood, urine, saliva and other biofluids. Some miRs found in these biofluids are sourced from specific tissues and may serve as biomarkers of tissue perturbation and early disease processes, including fatty liver disease and hepatotoxicity [3].

Polychlorinated biphenyls (PCBs) were manufactured from 1929-1971at a facility in Anniston, Alabama, and release of PCB-containing waste resulted in high local levels of environmental contamination. Concerns over the health effects of this contamination prompted the Agency for Toxic Substances Disease Registry (ATSDR) to partner with community members and university researchers to fund an exposure and health effects survey. The result was the Anniston Community Health Survey (ACHS) study cohort. TASH was previously associated with exposures to specific PCB congeners in ACHS [4]. We hypothesized that previously identified individuals with TASH in the ACHS study cohort will exhibit an altered liver miR profile in serum compared to those without TASH.

Materials and Methods

The original ACHS sample collection was carried out as a two-stage procedure previously described elsewhere [5-8]. Serum samples were archived at -80ºC until use for assays described below.

Serum levels of the liver injury biomarkers cytokeratin 18 M65 (CK18 M65; hepatocyte necrosis marker) and M30 (CK18 M30; hepatocyte apoptosis marker) were previously measured by ELISA (DiaPharma) in the ACHS cohort. Subjects were grouped by liver disease status according to predetermined CK18 levels as follows: “Not TASH” (M65 <300 U/L and M30 <200 U/L) and “TASH” (M65 >300 U/L and M30 <200 U/L) [4]. In this pilot study, 152 subjects were evaluated. Equal numbers (n=76) of “TASH” and “Not TASH” subjects were selected. The liver disease groups were stratified by sex so that each group had equal numbers of male and female subjects (n=38).

Preselected miRs (n=68) were profiled using the Firefly miRNA multiplex assay standard protocol (Abcam, Cambridge, UK) run on 50µl of archived serum for each sample.

Generalized linear models were constructed to calculate estimates of quantile-normalized log10-transformed mean fluorescent intensities (MFIs) for each liver disease group and to compare for statistical differences. All analyses used quantile-normalized non-background substituted MFI values and were corrected for measurement differences due to assay plate-to-plate differences.

To test for differences in level by liver disease group (TASH *vs*. not TASH), the following models were constructed: unadjusted (except for plate), and adjusted for epidemiology variables (age, race, and body mass index (BMI)) previously measured serum PCBs and lipids (log10-transformed sum of 35 ortho-substituted and log10-transformed total lipids). Associations between log10-transformed CK18 markers and target miRs were tested using generalized linear models which were adjusted for significant covariates. Statistics were previously performed as described [5-8, 10]. Multivariable analyses were false discovery rate (FDR)-corrected and considered statistically significant at the FDR p-value of ≤0.10 level or less. Analyses were performed using SAS v9.4 (Cary, NC).

Results and Discussion

Demographic information is provided in Table 1. Significant differences were seen between liver disease groups with respect to BMI, total lipids, and race/ethnicity. There were no significant intergroup differences with respect to age or ∑PCBs (whole weight), but the ∑PCBs was 1.66 times higher in the TASH group.

Characteristic	Liver disease status: Not TASH (n = 76)	Liver disease status: TASH (n = 76)	p-value
Age (years)	56.5±13.8	55.3±14.5	0.62
BMI (kg/m^2)	30.2±6.0	32.6±8.1	**0.05**
∑PCBs (whole weight)	6.8±8.6	11.3±26.6	0.16
Total lipids (mg/dL)	605.8±139.0	658.6±173.5	**0.04**
Race/ethnicity			**0.03**
Non-Hispanic White	31 (40.8)	44 (57.9)	
Nonwhite	45 (59.2)	32 (42.1)	

On univariate analysis, significant associations were seen between quantile-normalized miRs and age (n=17), BMI (n=10), race/ethnicity (n=15), and ∑PCBs (lipid adjusted, n=15). Six miRs were significantly enriched in the TASH group in adjusted models (FDR≤0.06). Of these, hsa-miR-122-5p exhibited the greatest fold difference between groups. Of note, the hsa-miR-192-5p expression pattern was most similar to hsa-miR-122-5p, which has been seen in previous studies of serum miR biomarkers in fatty liver disease progression [11-13]. Twelve miRs (including 5 of the 6 miRs which were differentially expressed in TASH) were significantly associated with CK18 M65 (raw $p<0.05$) in adjusted models (Fig. 1). A few miRs remained significantly associated with ∑PCB exposures (raw $p\leq0.06$) in adjusted models.

To our knowledge, these miR data are the first ever generated in an environmental hepatology study. A total of 13 unique miRs previously implicated in hepatotoxicity were associated with TASH and/or its biomarker, CK18 M65. These results suggest that the previous ACHS liver disease categorization procedure based on CK18 was effective. Thus, the true prevalence of liver disease in the ACHS cohort is likely to be high, although imaging or liver biopsies would be needed to confirm this result.

Mir-122-5p was the single miR which was most strongly associated with both TASH and CK18 M65. Mir-122-5p expression is liver-specific, and it is the most abundant hepatic miR [14]. Moreover, it has previously been associated with steatohepatitis [14]. Mir-122-5p appears to be a promising candidate biomarker for future environmental epidemiology research. Let-7d-5p was also associated with both ∑PCB exposures and CK18 M65. Future studies should determine if there are causal or mechanistic relationships between PCB exposures, miR expression, and TASH.

This abstract does not reflect EPA policy.

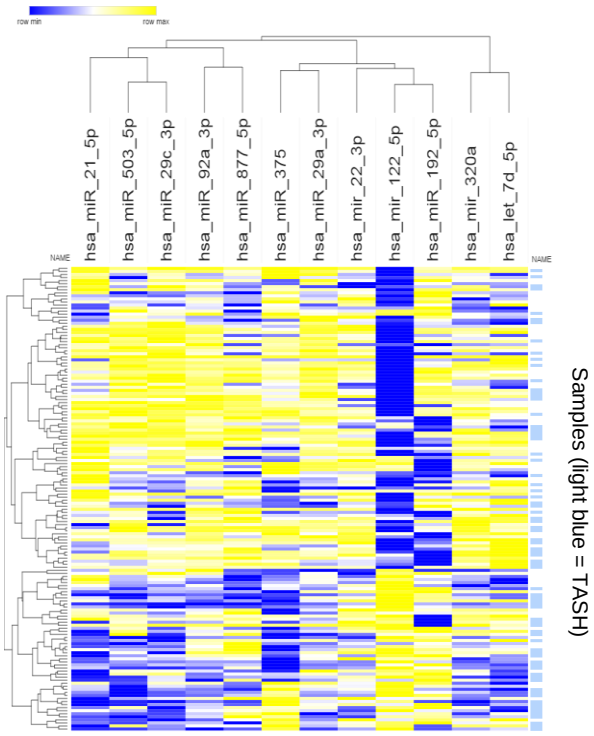

Fig. 1. Heatmap of miRs which were significantly associated with CK18 M65 in adjusted models. The heatmap shows the $\log_2$ fold-change values (of probe mean) of significant miRs. Probes and samples are grouped by hierarchal clustering (Spearman's ranking with complete linkage).

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