2333/ P209

Quantitative Dose-Response of Liver MicroRNA After Furan Exposure in Rodent Liver, Blood, and Cell Culture

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Objective and Background

Objective: To identify dose-responsive liver and blood miRNA markers associated with furan liver cytotoxicity and early tumorigenesis

Why Furan?

- Found in combustion by-products and food
- Nongenotoxic mouse liver carcinogen in NTP 2-year cancer bioassays and possible human carcinogen (IARC Group 2B)
- Established carcinogenic mode-of-action (MOA) involves chronic cytotoxicity and inflammation followed by regenerative proliferation; furan used as a reference chemical to determine early molecular biomarkers for this MOA
- Gene expression measurements of short-term exposure support this MOA and are predictive of cancer bioassay values

Why miRNA?

- Small non-coding RNA molecules (~22nt)
- Regulator of gene expression and translation
- Early and dose-responsive to environmental chemicals
- Tissue-specific differentially expressed miRNAs (DEmiRs) can be released into biofluids
- May have utility as early biomarkers of exposure and toxicity that link to furan's carcinogenic MOA

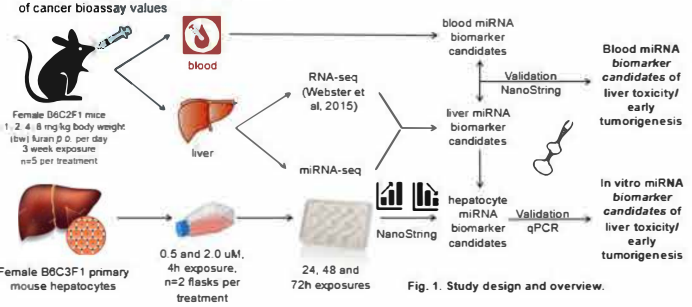

Fig. 1. Study design and overview.

Results: Liver

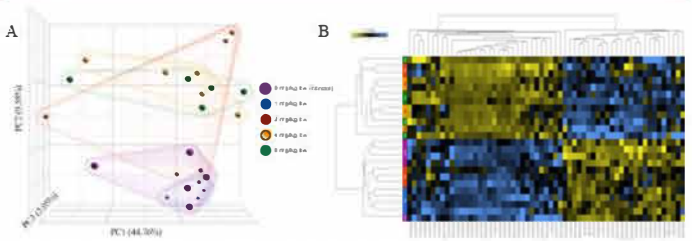

Fig. 2. Dose Separation after DESEQ2 analysis, liver DEmiRs filtered at FDR ≤ 0.05, FC ± 1.3. PCA of log2 normalized data (A) and hierarchal clustering of DEmiRs consistently altered at all three doses (B) show two clusters: (0 and 1 mg/kg doses) and (4 and 8 mg/kg doses) with the 2mg/kg dose overlapping both clusters.

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Results: Liver

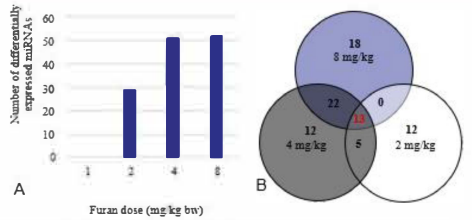

Fig. 3. Differentially Expressed Liver miRNAs, filtered at FDR ≤ 0.05, FC ± 1.3. Number of DEmiRs by treatment group (A), and Venn diagram of DEmiR overlap between treatment groups (B).

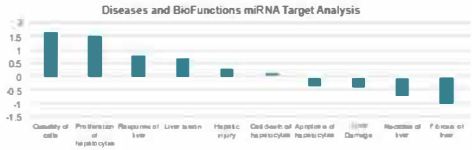

Fig. 4. Significantly altered (p < 0.05) Diseases and BioFunctions with z-scores for the carcinogenic dose (4 + 8 mg/kg) miRNA target analysis dataset.

Table 1. Liver miRNAs and target mRNAs (8 mg/kg) altered by furan exposure.

13 miRNAs significantly altered, 3 top doses	1mg/kg	2 mg/kg	4 mg/kg	8 mg/kg	Target mRNAs
mmu-miR-125a-5p*	1.01				
mmu-miR-30c-1-3p*	1.03				
mmu-miR-676-3p*	1.01				
mmu-miR-322-3p*	1.10				
mmu-miR-871-3p	1.01				
mmu-miR-2783	1.05				
mmu-miR-298-3p	1.01				
mmu-miR-17-5p*	1.13				
mmu-miR-203b-3p*	1.37				
mmu-miR-324-5p*	1.19				
mmu-miR-148b-3p	1.22				
mmu-miR-34a-5p*	1.35				

* miRNAs exhibiting a robust dose-response trend in BMD Express

\# miRNAs confirmed by Nano String

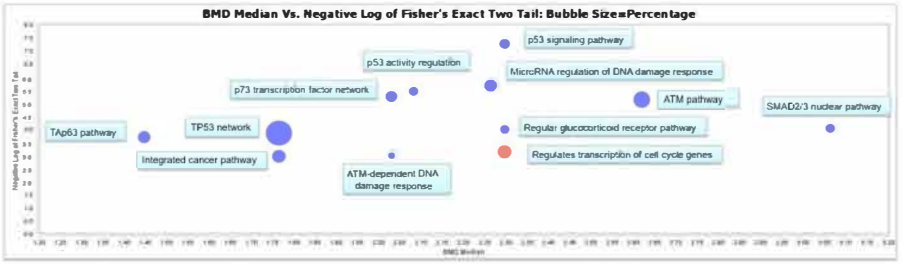

Fig. 5. BMDExpress pathways analyses. Significant dose-responsive (p ≤ 0.001) Reactome (red) and Bioplanet (blue) pathways for IPA-identified miRNA targets.

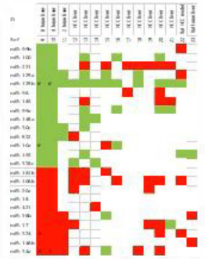

Fig. 6. Overlap of furan DEmiRs with HCC DEmiRs from the literature.

Dose-responsive liver and blood miRNA biomarkers indicate furan-mediated liver cytotoxicity and tumorigenic mode-of-action

Results: Blood and Cell Culture

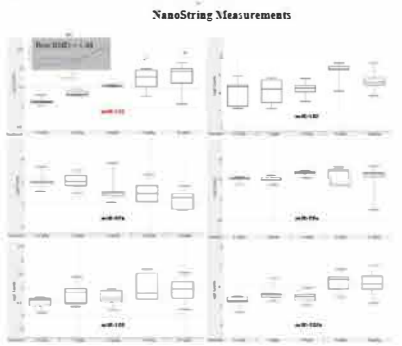

Fig. 7. Dose-responsive blood miRNA measured by NanoString and BMD modeling. Only miR-122 is statistically significant and was also significantly upregulated in the liver at the 2 mg/kg bw dose only. Several other miRNAs trend in the same (-99b, -203b) or opposite (-183, -99a, -100) direction as their liver counterparts, all of which were significantly altered at both 4 and 8 mg/kg bw.

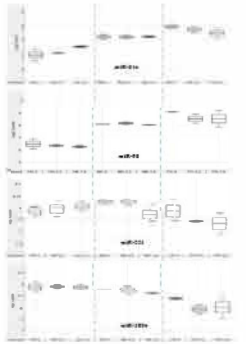

Fig. 8. Measurement of selected cell lysate liver biomarker candidates by NanoString. None were statistically significant (n=2); however, there are some dose-related trends evident. miR-34a was also measured by qPCR and trended downward at 72h.

Summary and Conclusions

- Liver miRNA expression differed between 4 and 8 mg/kg bw treatment profiles and 0 and 1 mg/kg bw treatment profiles, with the 2 mg/kg profile spanning both.
- 35 liver miRNA were significantly altered at both carcinogenic doses (4 and 8 mg/kg bw). Functional analysis of the dose-responsive miRNA targets indicated miRNA involvement in p53 signaling, cell cycle and DNA damage response. An increase in hepatocyte proliferation and a decrease in apoptosis were indicated.
- These data support previous studies that demonstrated cellular response to toxicity and proliferation are occurring in the mouse liver after short term exposure to furan at these doses.
- miRNA appear to be more sensitive early indicators of furan toxicity than mRNA, with lower BMD values.
- Many of the altered liver miRNA have been associated with HCC in the literature.
- Some of these liver toxicity miRNA biomarker candidates were also altered in the blood and may thereby serve as blood biomarkers of early key events in liver tumorigenesis (miR-183, miR-203b, miR-122).
- Preliminary evidence suggests that primary hepatocyte culture may be a useful tool for miRNA biomarker discovery.

Our results indicate mechanistic involvement of miRNA in furan tumorigenicity and identify several candidates with potential utility as accessible, dose-responsive biomarkers of chemical-mediated disease outcome.