

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

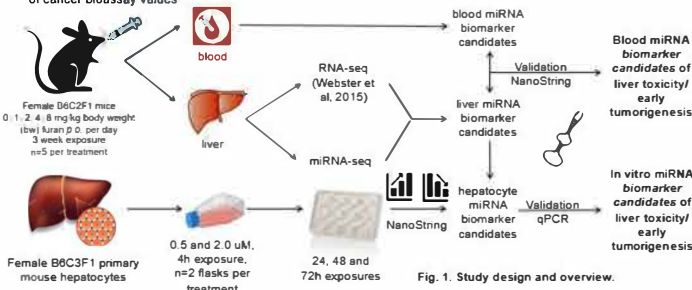


Fig. 1. Study design and overview

Results: Liver

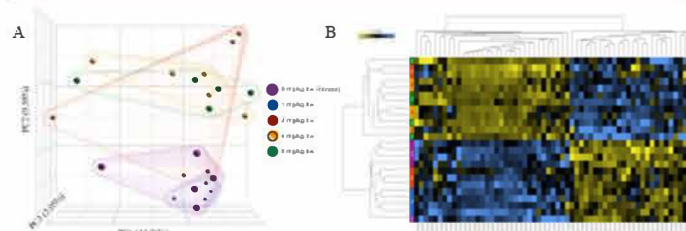


Fig. 2. Dose Separation after DESEQ2 analysis, liver DEmiRs filtered at $FDR \leq 0.05$, $FC \pm 1.3$. PCA of log2 normalized data (A) and hierarchical clustering of DEmiRs consistently altered at all three doses (B) show two clusters: (0 and 1 mg/kg doses) and (4 and 8 mg/kg doses) with the 2mg/kg dose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

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Results: Liver

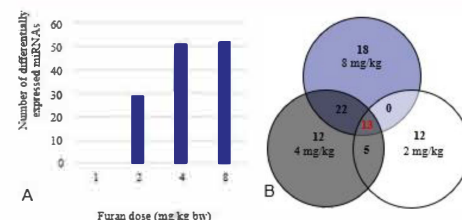


Fig. 3. Differentially Expressed Liver miRNAs, filtered at $FDR \leq 0.05$, $FC \geq 1.3$. Number of DE miRs by treatment group (A), and Venn diagram of DE miR overlap between treatment groups (B).

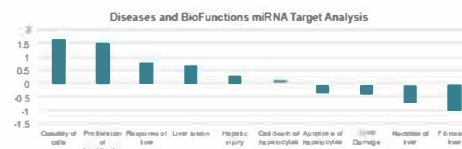


Fig. 4. Significantly altered ($p < 0.05$) Diseases and BioFunctions with z-scores for the carcinogenic dose (4 + 8 mg/kg) miRNA target analysis dataset

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

13 miRNAs significantly altered at 4 h post-dose	1 miR/g	2 miR/g	4 miR/g	8 miR/g	Target mRNAs
mmu-miR-123a-5p	1.03	0.78	0.83	1.38	Adm1c1, Ahr, Ahn1, Ahn2, Cyp1b1, Cyp1b2, Cyp1b3, Cyp1b4, Cyp1b5, Cyp1b6, Cyp1b7, Cyp1b8, Cyp1b9, Cyp1b10, Cyp1b11, Cyp1b12, Cyp1b13, Cyp1b14, Cyp1b15, Cyp1b16, Cyp1b17, Cyp1b18, Cyp1b19, Cyp1b20, Cyp1b21, Cyp1b22, Cyp1b23, Cyp1b24, Cyp1b25, Cyp1b26, Cyp1b27, Cyp1b28, Cyp1b29, Cyp1b30, Cyp1b31, Cyp1b32, Cyp1b33, Cyp1b34, Cyp1b35, Cyp1b36, Cyp1b37, Cyp1b38, Cyp1b39, Cyp1b40, Cyp1b41, Cyp1b42, Cyp1b43, Cyp1b44, Cyp1b45, Cyp1b46, Cyp1b47, Cyp1b48, Cyp1b49, Cyp1b50, Cyp1b51, Cyp1b52, Cyp1b53, Cyp1b54, Cyp1b55, Cyp1b56, Cyp1b57, Cyp1b58, Cyp1b59, Cyp1b60, Cyp1b61, Cyp1b62, Cyp1b63, Cyp1b64, Cyp1b65, Cyp1b66, Cyp1b67, Cyp1b68, Cyp1b69, Cyp1b70, Cyp1b71, Cyp1b72, Cyp1b73, Cyp1b74, Cyp1b75, Cyp1b76, Cyp1b77, Cyp1b78, Cyp1b79, Cyp1b80, Cyp1b81, Cyp1b82, Cyp1b83, Cyp1b84, Cyp1b85, Cyp1b86, Cyp1b87, Cyp1b88, Cyp1b89, Cyp1b90, Cyp1b91, Cyp1b92, Cyp1b93, Cyp1b94, Cyp1b95, Cyp1b96, Cyp1b97, Cyp1b98, Cyp1b99, Cyp1b100, Cyp1b101, Cyp1b102, Cyp1b103, Cyp1b104, Cyp1b105, Cyp1b106, Cyp1b107, Cyp1b108, Cyp1b109, Cyp1b110, Cyp1b111, Cyp1b112, Cyp1b113, Cyp1b114, Cyp1b115, Cyp1b116, Cyp1b117, Cyp1b118, Cyp1b119, Cyp1b120, Cyp1b121, Cyp1b122, Cyp1b123, Cyp1b124, Cyp1b125, Cyp1b126, Cyp1b127, Cyp1b128, Cyp1b129, Cyp1b130, Cyp1b131, Cyp1b132, Cyp1b133, Cyp1b134, Cyp1b135, Cyp1b136, Cyp1b137, Cyp1b138, Cyp1b139, Cyp1b140, Cyp1b141, Cyp1b142, Cyp1b143, Cyp1b144, Cyp1b145, Cyp1b146, Cyp1b147, Cyp1b148, Cyp1b149, Cyp1b150, Cyp1b151, Cyp1b152, Cyp1b153, Cyp1b154, Cyp1b155, Cyp1b156, Cyp1b157, Cyp1b158, Cyp1b159, Cyp1b160, Cyp1b161, Cyp1b162, Cyp1b163, Cyp1b164, Cyp1b165, Cyp1b166, Cyp1b167, Cyp1b168, Cyp1b169, Cyp1b170, Cyp1b171, Cyp1b172, Cyp1b173, Cyp1b174, Cyp1b175, Cyp1b176, Cyp1b177, Cyp1b178, Cyp1b179, Cyp1b180, Cyp1b181, Cyp1b182, Cyp1b183, Cyp1b184, Cyp1b185, Cyp1b186, Cyp1b187, Cyp1b188, Cyp1b189, Cyp1b190, Cyp1b191, Cyp1b192, Cyp1b193, Cyp1b194, Cyp1b195, Cyp1b196, Cyp1b197, Cyp1b198, Cyp1b199, Cyp1b200, Cyp1b201, Cyp1b202, Cyp1b203, Cyp1b204, Cyp1b205, Cyp1b206, Cyp1b207, Cyp1b208, Cyp1b209, Cyp1b210, Cyp1b211, Cyp1b212, Cyp1b213, Cyp1b214, Cyp1b215, Cyp1b216, Cyp1b217, Cyp1b218, Cyp1b219, Cyp1b220, Cyp1b221, Cyp1b222, Cyp1b223, Cyp1b224, Cyp1b225, Cyp1b226, Cyp1b227, Cyp1b228, Cyp1b229, Cyp1b230, Cyp1b231, Cyp1b232, Cyp1b233, Cyp1b234, Cyp1b235, Cyp1b236, Cyp1b237, Cyp1b238, Cyp1b239, Cyp1b240, Cyp1b241, Cyp1b242, Cyp1b243, Cyp1b244, Cyp1b245, Cyp1b246, Cyp1b247, Cyp1b248, Cyp1b249, Cyp1b250, Cyp1b251, Cyp1b252, Cyp1b253, Cyp1b254, Cyp1b255, Cyp1b256, Cyp1b257, Cyp1b258, Cyp1b259, Cyp1b260, Cyp1b261, Cyp1b262, Cyp1b263, Cyp1b264, Cyp1b265, Cyp1b266, Cyp1b267, Cyp1b268, Cyp1b269, Cyp1b270, Cyp1b271, Cyp1b272, Cyp1b273, Cyp1b274, Cyp1b275, Cyp1b276, Cyp1b277, Cyp1b278, Cyp1b279, Cyp1b280, Cyp1b281, Cyp1b282, Cyp1b283, Cyp1b284, Cyp1b285, Cyp1b286, Cyp1b287, Cyp1b288, Cyp1b289, Cyp1b290, Cyp1b291, Cyp1b292, Cyp1b293, Cyp1b294, Cyp1b295, Cyp1b296, Cyp1b297, Cyp1b298, Cyp1b299, Cyp1b300, Cyp1b301, Cyp1b302, Cyp1b303, Cyp1b304, Cyp1b305, Cyp1b306, Cyp1b307, Cyp1b308, Cyp1b309, Cyp1b310, Cyp1b311, Cyp1b312, Cyp1b313, Cyp1b314, Cyp1b315, Cyp1b316, Cyp1b317, Cyp1b318, Cyp1b319, Cyp1b320, Cyp1b321, Cyp1b322, Cyp1b323, Cyp1b324, Cyp1b325, Cyp1b326, Cyp1b327, Cyp1b328, Cyp1b329, Cyp1b330, Cyp1b331, Cyp1b332, Cyp1b333, Cyp1b334, Cyp1b335, Cyp1b336, Cyp1b337, Cyp1b338, Cyp1b339, Cyp1b340, Cyp1b341, Cyp1b342, Cyp1b343, Cyp1b344, Cyp1b345, Cyp1b346, Cyp1b347, Cyp1b348, Cyp1b349, Cyp1b350, Cyp1b351, Cyp1b352, Cyp1b353, Cyp1b354, Cyp1b355, Cyp1b356, Cyp1b357, Cyp1b358, Cyp1b359, Cyp1b360, Cyp1b361, Cyp1b362, Cyp1b363, Cyp1b364, Cyp1b365, Cyp1b366, Cyp1b367, Cyp1b368, Cyp1b369, Cyp1b370, Cyp1b371, Cyp1b372, Cyp1b373, Cyp1b374, Cyp1b375, Cyp1b376, Cyp1b377, Cyp1b378, Cyp1b379, Cyp1b380, Cyp1b381, Cyp1b382, Cyp1b383, Cyp1b384, Cyp1b385, Cyp1b386, Cyp1b387, Cyp1b388, Cyp1b389, Cyp1b390, Cyp1b391, Cyp1b392, Cyp1b393, Cyp1b394, Cyp1b395, Cyp1b396, Cyp1b397, Cyp1b398, Cyp1b399, Cyp1b400, Cyp1b401, Cyp1b402, Cyp1b403, Cyp1b404, Cyp1b405, Cyp1b406, Cyp1b407, Cyp1b408, Cyp1b409, Cyp1b410, Cyp1b411, Cyp1b412, Cyp1b413, Cyp1b414, Cyp1b415, Cyp1b416, Cyp1b417, Cyp1b418, Cyp1b419, Cyp1b420, Cyp1b421, Cyp1b422, Cyp1b423, Cyp1b424, Cyp1b425, Cyp1b426, Cyp1b427, Cyp1b428, Cyp1b429, Cyp1b430, Cyp1b431, Cyp1b432, Cyp1b433, Cyp1b434, Cyp1b435, Cyp1b436, Cyp1b437, Cyp1b438, Cyp1b439, Cyp1b440, Cyp1b441, Cyp1b442, Cyp1b443, Cyp1b444, Cyp1b445, Cyp1b446, Cyp1b447, Cyp1b448, Cyp1b449, Cyp1b450, Cyp1b451, Cyp1b452, Cyp1b453, Cyp1b454, Cyp1b455, Cyp1b456, Cyp1b457, Cyp1b458, Cyp1b459, Cyp1b460, Cyp1b461, Cyp1b462, Cyp1b463, Cyp1b464, Cyp1b465, Cyp1b466, Cyp1b467, Cyp1b468, Cyp1b469, Cyp1b470, Cyp1b471, Cyp1b472, Cyp1b473, Cyp1b474, Cyp1b475, Cyp1b476, Cyp1b477, Cyp1b478, Cyp1b479, Cyp1b480, Cyp1b481, Cyp1b482, Cyp1b483, Cyp1b484, Cyp1b485, Cyp1b486, Cyp1b487, Cyp1b488, Cyp1b489, Cyp1b490, Cyp1b491, Cyp1b492, Cyp1b493, Cyp1b494, Cyp1b495, Cyp1b496, Cyp1b497, Cyp1b498, Cyp1b499, Cyp1b500, Cyp1b501, Cyp1b502, Cyp1b503, Cyp1b50

BMD Median Vs. Negative Log of Fisher's Exact Two Tail; Bubble Size=Percentage

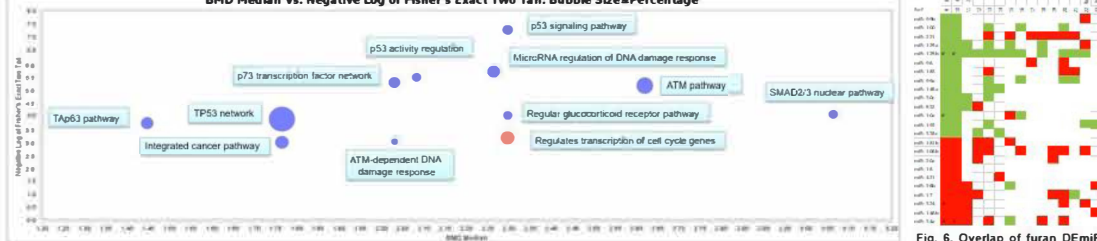


Fig. 5. BMDExpress pathways analyses. Significant dose-responsive ($p < 0.001$) Reactome (red) and Bioplanet (blue) pathways for IPA-identified miRNA targets

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

Results: Blood and Cell Culture

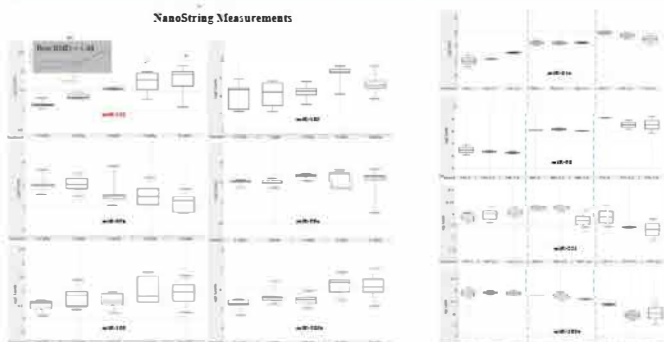


Fig. 7. Dose-response of *miR-122* mRNA measured by NanoString and BMD models. Only *miR-122* statistically significant in both models. *miR-122* was also significantly upregulated in the liver at the 2 mg/kg bw dose only. Several other miRNAs trend in the same (-90b, -203b) or opposite (-183, -90a, -100) direction as their liver counterparts, all of which were significantly altered at both 4 and 8 mg/kg bw.

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.