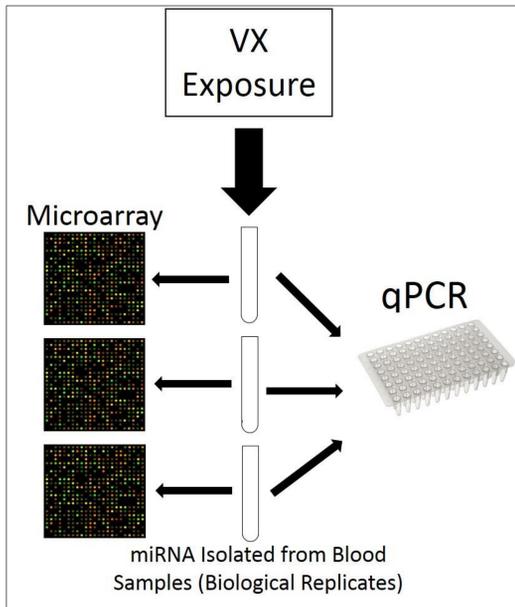


Background

Chemical warfare agents continue to be a threat to military and civilian populations at home and abroad during the war against terrorism. Exposure to chemical nerve agents such as VX is known to disrupt cholinergic pathways, resulting in a cascade of toxic effects relative to the exposure level, including miosis, excessive secretions, convulsions, seizures, and death. While much is known about the main effects of nerve agents, there remains a paucity of information about the molecular effects. MicroRNAs are a relatively novel epigenetic regulator of gene expression. Each mature miRNA can potentially regulate hundreds of mRNA targets, and therefore affect multiple disparate biological functions. This investigation, as part of the larger Systems Biology of Host-Toxicant Response project, is aimed at: discovery and validation of novel biomarkers of VX exposure and effect.

Biomarkers for early time points are key for detect-to-treat CONOPs; later time points are useful for forensic CONOPs.

Methods



Animal Samples

•Whole blood from adult male Sprague-Dawley rats was collected at 1, 2, 4, 8, or 24 hours after exposure to 0.4, 0.7, or 1.0 x LD₅₀ VX (*i.v.*). Total RNA was isolated.

Expression Analysis

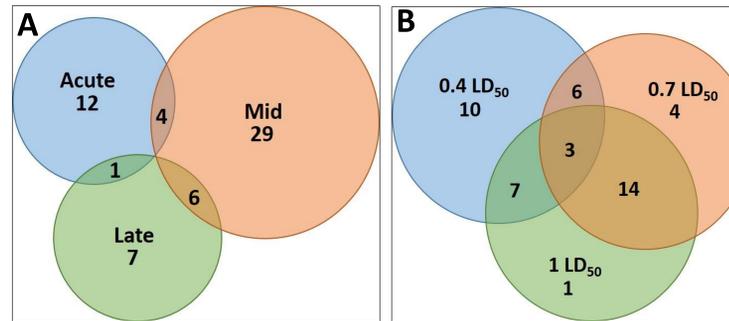
Microarray: Samples were processed for microRNA hybridization on Affymetrix miRNA 3.1 array plates.
qPCR: Samples were pooled and processed for qPCR with QIAGEN miScript miRNome V16.

Acknowledgements

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Approved for Public Release

miRNA Expression Overview



Microarray results for miRNA expression were normalized via Affymetrix software. Statistical significance was determined by Student t-test ($p < 0.05$). miRNAs with a fold-change ≥ 1.2 or ≤ 0.8 at any dose or time point are presented above ($n = 54$).

Panel A: miRNAs for all LD₅₀ values were grouped into acute (1h and 2h), mid (4h and 8h) or late (24h) groups.

Panel B: miRNAs for all time values were grouped into 0.4 LD₅₀, 0.7 LD₅₀, or 1 LD₅₀ groups.

miRNA Biological Function

A	Acute	Mid	Late	Pro-apoptotic Gene Targets	Anti-apoptotic Gene Targets
	rno-miR-146a-5p		↓	↓	FAS
rno-miR-122-5p	↑				IGF1R, BCL2L2
rno-miR-133b-3p		↓	↓	TLR2, FADD, BRCA1, CDKN1A	BCL2L2, MCL1, IGF1R, FAIM, NFKB1, CD40LG
rno-miR-143-3p	↑				
rno-miR-30e-5p			↓		
rno-miR-322-3p	↑	↓			
rno-miR-409a-3p			↓		

B	0.4 LD ₅₀	0.7 LD ₅₀	1 LD ₅₀	Arrhythmogenic Gene Targets
	rno-miR-192-5p	↑	↑	
rno-miR-133a-3p		↓	↓	CACNA1C
rno-miR-208a-3p	↑			
rno-miR-30c-1-3p			↓	
rno-miR-30e-5p			↓	

Statistically significant miRNAs via microarray ($p > 0.05$; above) were analyzed for potential biological relevance. **Table A:** Pro-apoptotic miRNAs are up-regulated during acute VX exposure (1h and 2h timepts), but down-regulated at later time pts. Pro-apoptotic and anti-apoptotic

experimentally identified mRNA targets are listed for each miRNA. TLR2 is up-regulated at acute and mid time pts via mRNA microarray analysis of VX-treated samples (data not shown). **Table B:** Cardiac arrhythmias are a symptom of organophosphate exposure. miRNAs experimentally identified to be regulated during cardiac arrhythmias are grouped by VX dose, showing upregulation during low doses and downregulation during higher doses.

Biomarker Panels

Timept	Panel	Accuracy	FP	FN
1hr	miR-133a-3p, miR-1188-3p, miR-208a-3p, miR-346	95%	0	1-2
2hr	miR-133a-3p, miR-352, miR-99a, miR-205	86%	1	4-6
4hr	miR-133b-3p, miR-346, miR-466c-3p, miR-205	85%	2-3	4-5
8hr	miR-28-5p, miR-30c-1-3p, miR-346, miR-342-5p, miR-99b-5p	95%	1	1-3
24hr	miR-208a-3p, miR-1188-3p, miR-335, miR-99b-5p	88%	0	5-6

Biomarker panels were determined from microarray results using Support Vector Machine with a radial basis function kernel. At each timept, 0.4, 0.7, and 1 LD₅₀ samples were combined as the exposed class ($n = 18$). All controls at all time pts were combined as the unexposed class ($n = 25$). Accuracy was calculated for each panel, as well as false positives (FP) and false negatives (FN).

Diagnostic Detection

miRNA	1hr	2hr	4hr	8hr	24hr
miR-133a-3p	↓	↑↓			
miR-133b-3p			↓		
miR-1188-3p	↑				↓
miR-28-5p				↓	
miR-205		↓	↓		
miR-208a-3p	↑				↓
miR-30c-1-3p				↓	
miR-335					↓
miR-342-5p				↓	
miR-346	↓		↓	↓	
miR-352		↑			
miR-466c-3p			↓		
miR-99a		↓			
miR-99b-5p				↓	↑

Panels were compared to qPCR results. Panel trends for each miRNA and timept are listed with arrows. Cells are color coded for PCR concurrence: **Green** = concurrence; **Orange** = no change in qPCR; **Yellow** = no qPCR data; **Red** = Opposite trend in qPCR.

- miRNA biomarker panels can predict VX exposure at the 1 hour and 24 hour time points with no false positives
- Microarray-derived miRNA panels correlate with qPCR at early time points, with predicted concurrence.