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Global transcriptional analysis of short-term hepatic stress responses in Atlantic salmon (*Salmo salar*) exposed to depleted uranium



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ABSTRACT

Potential environmental hazards of radionuclides are often studied at the individual level. Sufficient toxicogenomics data at the molecular/cellular level for understanding the effects and modes of toxic action (MoAs) of radionuclide is still lacking. The current article introduces transcriptomic data generated from a recent ecotoxicological study, with the aims to characterize the MoAs of a metallic radionuclide, deplete uranium (DU) in an ecologically and commercially important fish species, Atlantic salmon (*Salmo salar*). Salmon were exposed to three concentrations (0.25, 0.5 and 1.0 mg/L) of DU for 48 h. Short-term global transcriptional responses were studied using Agilent custom-designed high density 60,000-feature (60 k) salmonid oligonucleotide microarrays (oligoarray). The microarray datasets deposited at Gene Expression Omnibus (GEO ID: GSE58824) were associated with a recently published study by Song et al. (2014) in BMC Genomics. The authors describe the experimental data herein to build a platform for better understanding the toxic mechanisms and ecological hazard of radionuclides such as DU in fish.

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Specifications	
Organism/cell line/tissue	Salmo salar
Sex	Juvenile (parr)
Sequencer or array type	Agilent 60,000-feature (60 k) custom oligonucleotide salmonid microarray
Data format	Raw and normalized
Experimental factors	Control (pure lake water) versus 0.25, 0.5 and 1.0 mg/L depleted uranium (DU).
Experimental features	The single-color microarray analysis was performed using total RNA isolated from liver tissue of Atlantic salmon after 48 h waterborne exposure to different concentrations of DU.
Consent	n/a
Sample source location	Oslo, Norway

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58824

Experimental design, materials and methods

Fish exposure

To study the short-term effects of waterborne depleted uranium (DU), Atlantic salmon parr (N = 6) were exposed for 48 h to 0, 0.25, 0.5 and 1.0 mg/L uranyl acetate $(UO_2(CH_3COO)_2 \cdot 2H_2O)$, purity \geq 98.0%, specific activity 1.459×10^4 Bq/g, Fluka, Sigma-Aldrich, Buchs, Switzerland) dissolved in lake water collected from Lake Maridalsvannet, Oslo, Norway. Fish experiments were approved by the Norwegian Animal Research Authority (NARA ID: 3026) and conducted at the FIGARO facility for environmental radioactivity studies (Norwegian University of Life Sciences, Ås, Norway). All operations strictly followed the Norwegian Welfare Act and research animal legislation.

Tissue collection and RNA isolation

Immediately after the exposure, fish were terminated by cephalic concussion. Liver tissue was dissected in snap-frozen in liquid nitrogen. Samples were then stored in ultrafreezer (-80 °C) until further analysis.

For RNA isolation, the RNeasy Plus Mini kit (Qiagen, Hilden, Germany) was used to extract total RNA from 20 to 30 mg frozen liver. The procedures have been previously described in detail [1,2]. The RNA

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Table 1	
Quality assessment for microarray raw data	

Sample ID (GEO) ^a	Treatment	Non-uniformed outlier features (%)	# of good QC metrics (total 10)	Correlation of Agilent SpikeIn controls ^b (R ²)
GSM1420181	Control 1	0	8	0.99
GSM1420182	Control 2	0.04	9	0.98
GSM1420183	Control 3	0.05	8	0.99
GSM1420184	0.25 mg U/L 1	0.03	8	0.99
GSM1420185	0.25 mg U/L 2	0.03	9	0.99
GSM1420186	0.25 mg U/L 3	0	9	0.99
GSM1420187	0.5 mg U/L 1	0.01	9	0.99
GSM1420188	0.5 mg U/L 2	0	8	0.99
GSM1420189	0.5 mg U/L 3	0	8	0.99
GSM1420190	1.0 mg U/L 1	0	9	0.99
GSM1420191	1.0 mg U/L 2	0	9	0.99
GSM1420192	1.0 mg U/L 3	0.03	8	0.99

^a GEO: Gene Expression Omnibus.

^b Agilent Spikeln control: Internal standard for quality assessment of hybridization (Agilent Technologies).

yield and purity (yield > 200 ng/µL, 260/230 > 2.0, 260/280 > 1.8) were determined using Nanodrop[®] spectrophotometer (ND-1000, Nanodrop Technologies, Wilminton, Delaware, USA). The RNA integrity (RIN > 9.0) was determined using Bioanalyzer RNA 6000 Nano chips (Agilent technologies, Santa Clara, California, USA) following the manufacturer's manual.

Microarray design and hybridization

The microarray probes (totally 55,418 features) were designed using the consensus sequences of two salmonid fish, *Salmo salar* and *Oncorhynchus mykiss* from the cGRASP 44 k salmonid oligoarray (35,920 sequences), Release 11/09 [3] complimented by NCBI Unigenes (2995 sequences from *S. salar*, build 31 and 16503 sequences from *O. mykiss*, build 27) [4]. The cross-hybridization potential of the array probes was predicted to be less than 7% (3587 probes). A high density 60,000-feature (60 k) custom salmonid oligonucleotide array was then manufactured by Agilent Technologies (Santa Clara, CA, USA). More detailed information can be found in the previous publication by this research group [2]. The custom salmonid array platform is currently available in Gene Expression Omnibus (GEO ID: GPL18864).

The Agilent "One-Color Microarray-Based Gene Expression Analysis (v6.5)" protocol (Agilent Technologies) was used in the microarray analysis with small modifications [2]. Two hundred nanogram of liver total RNA was used as input material for array experiment (N = 3). The hybridized array slides were scanned using Agilent microarray c scanner (Agilent Technologies, scan region: 61×21.6 mm, resolution: 3 µm, output Tiff image: 20 bit).

Data collection and processing

The Agilent Feature Extraction (FE) software (v10.7) was used to extract raw data from scanned array images. The quality assessment for extracted data was based on the quality control (QC) files generated by the FE software (Table 1) and correlations of signal intensity of control probes between different arrays. High quality raw data (signal intensity values) was further processed using GeneSpring software (v11.0, Agilent Technologies). Briefly, raw data were first corrected for background signals, flagged for low quality and missing features and then normalized within- and between-array using 75% quantile method. Values from replicate features were computed for median and merged to a single normalized signal intensity value for each RNA source sequence. After raw data processing, in total 40,267 features were log-2 transformed and used for downstream statistical analysis to determine differentially expressed gene transcripts (DEGs).

Discussion

This article describes high quality transcriptomic datasets generated from an ecotoxicological study on the early stress responses in Atlantic salmon after short-term (48 h) exposure to waterborne depleted uranium (DU) using a custom microarray for salmonid fish. The full study has recently been published [2] and these datasets may serve as a platform to understand the toxic mechanisms and ecological hazard of environmental radionuclides such as DU in fish.

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- [3] cGRASP, http://www.cgrasp.org/.
- [4] Unigene, http://www.ncbi.nlm.nih.gov/unigene.