Environmental Toxicology and Pharmacology 56 (2017) 366–374

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Research Paper

Estrogen-responsive gene networks in the teleost liver: What are the key molecular indicators?

April Feswick[b], Kelly R. Munkittrick[c], Christopher J. Martyniuk[a,b,*]

[a] Department of Physiological Sciences and Center for Environmental and Human Toxicology, UF Genetics Institute, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611, USA

[b] Department of Biology, University of New Brunswick, Saint John, New Brunswick E2L 4L5, Canada

[c] Executive Director of Cold Regions and Water Initiatives, Wilfred Laurier University

ARTICLE INFO

Keywords:
Network analysis
Endocrine disruptors
Computational biology
Environmental toxicology
Teleost

ABSTRACT

An overarching goal of environmental genomics is to leverage sensitive suites of markers that are robust and reliable to assess biological responses in a range of species inhabiting variable environments. The objective of this study was to identify core groups of transcripts and molecular signaling pathways that respond to 17alpha-ethylinestadiol (EE2), a ubiquitous estrogenic contaminant, using transcriptome datasets generated from six independent laboratories. We sought to determine which biomarkers and gene networks were those most robust and reliably detected in multiple laboratories. Six laboratories conducted microarray analysis in pieces of the same liver from male fathead minnows exposed to ~15 ng/L EE2 for 96 h. There were common transcriptional networks identified in every dataset. These included down-regulation of gene networks associated with blood clotting, complement activation, triglyceride storage, and xenobiotic metabolism. Noteworthy was that more than ~85% of the gene networks were suppressed by EE2. Leveraging both these data and those mined from the Comparative Toxicogenomics Database (CTD), we narrowed in on an EE2-responsive transcriptional network. All transcripts in this network responded ~ ± 5-fold or more to EE2, increasing reliability of detection. This network included estrogen receptor alpha, transferrin, myeloid cell leukemia 1, insulin like growth factor 1, insulin like growth factor binding protein 2, and methionine adenosyltransferase 2A. This estrogen-responsive interactome has the advantage over single markers (e.g. vitellogenin) in that these entities are directly connected to each other based upon evidence of expression regulation and protein binding. Thus, it represents an interacting functional suite of estrogenic markers. Vitellogenin, the gold standard for estrogenic exposures, can show high individual variability in its response to estrogens, and the use of a multi-gene approach for estrogenic chemicals is expected to improve sensitivity. In our case, the coefficient of variation was significantly lowered by the gene network (~67%) compared to Vtg alone, supporting the use of this transcriptional network as a sensitive alternative for detecting estrogenic effluents and chemicals. We propose that screening chemicals for estrogenicity using interacting genes within a defined expression network will improve sensitivity, accuracy, and reduce the number of animals required for endocrine disruption assessments.

1. Introduction

Biological responses in aquatic organisms to both single and complex mixtures of chemicals have been quantified using a variety of omics-based technologies (Bahamonde et al., 2015; Martyniuk et al., 2012; Simmons et al., 2015), all of which have improved our understanding as to how aquatic organisms interface with their chemical environment. In order to conceptualize these "Big Data", bioinformatics methods that include gene set enrichment (Shi and Walker, 2007; Subramanian et al., 2005) and pathway analysis (Garcia-Reyero and Perkins, 2011) are used in ecotoxicology to synthesize molecular data into a biological interactome. Due to the significant number of studies in ecotoxicology using omics methods, detailed descriptions of molecular interactions are feasible within a biological network, generating relationships that may prove useful for monitoring adverse responses in complex environments.

A challenge for the wider acceptance of omics in risk assessment and environmental monitoring programs has been the lack of data demonstrating both reliability and consistency of omics data generated in the hands of multiple users. Studies examining inter-laboratory variability

* Corresponding author at: Department of Physiological Sciences and Center for Environmental and Human Toxicology, UF Genetics Institute, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611, USA.

E-mail address: cmartyn@ufl.edu (C.J. Martyniuk).

http://dx.doi.org/10.1016/j.etap.2017.10.012
Received 24 October 2017; Accepted 28 October 2017
Available online 31 October 2017
1382-6689/ © 2017 Elsevier B.V. All rights reserved.

using microarray data reveal that it is possible to detect the same differentially expressed transcripts in different laboratories, thus there is some congruence in laboratories for treatment-specific effects (Feswick et al., 2017; Hockley et al., 2009; Vidal-Dorsch et al., 2015). However, these studies also confirm the importance of standardization of methods, including bioinformatics approaches, wherever feasible. Moreover, technical variability and an array of statistical designs make cross-laboratory comparisons difficult. As such, there is debate as to whether or not these data are robust enough to be used in a regulatory context (Bahamonde et al., 2016), and it is clear that variability in data remains high enough to warrant caution in the interpretation of the results. For example, Vidal-Dorsch et al. (2015); Feswick et al. (2017) acknowledged that data were perhaps best interpreted when considering only those genes that were consistently identified as differentially expressed by different laboratories (i.e. a gene network approach). The challenge has been to determine whether data from any particular laboratory is reproducible without doing an *a priori* interlaboratory comparison for every experiment. However, as a research community, we have reached a critical mass of information that allows us to retrospectively hone in on central transcripts and pathways responsive to chemicals.

Inter-laboratory reproducibility has been acknowledged to be highest when data analysis focused on identifying biological themes defined by enriched Gene Ontology (GO) categories (Bammler et al., 2005; Beyer et al., 2007; Zhang et al., 2013), and not based on gene-by-gene comparisons. Thus, despite the variation present in transcriptomic data, and regardless of the cause (i.e. technical, biological), the use of hierarchical clustering is expected to reduce this variability and increase confidence in the interpretation of the data. Moreover, the use of enrichment software is immensely helpful for the biological interpretation of omics data, and for the discovery of underlying molecular mechanisms which drive the organismal response to chemical stressors. For example, gene networks have been useful in characterizing the ontogeny of human diseases, and in identifying specific molecular signatures for neurodegeneration (Wang et al., 2016) and cancers (Liu et al., 2015).

For these reasons, *a priori* identification of robust transcriptional networks that are consistently identified as biologically responsive to a given condition (i.e. hypoxia, chemicals) are expected to be more informative than either the use of single biomarkers or genome-wide transcriptional data. The use of a multi-gene network approach is expected to improve sensitivity by detecting subtle chemical exposures because they reduce dependence on a single biomarker to quantify the exposure. Drawbacks of using a single biomarker can be exemplified by the egg yolk precursor protein vitellogenin (Vtg): although it is currently the most widely measured bioindicator of estrogenic exposure (Folmar et al., 1996; Mellanen et al., 1999; Sumpter and Jobling, 1995) in aquatic species, high individual variability in the expression of Vtg makes the fidelity of such an estrogenic biomarker challenging at times. For example, Biales et al. (Biales et al., 2007) tested the expression of *vtg* in fathead minnows (FHM) following a 48 h exposure to 2.5 nM 17alpha-ethinylestradiol, and determined that 24 individuals were required to statistically discriminate between control and treatment groups with a power of 80%. This was attributed to the high variability in individual response, and the coefficient of variation of the normalized quantities ranged from 15 to 50% across individuals. Feswick et al. (2017) showed that the magnitude of Vtg response in FHM liver to 17alpha-ethinylestradiol in six laboratories varied significantly, with fold change estimates for Vtg1 and Vtg3 ranging between 10 and 1000 fold depending on the laboratory. Thus, accurately quantifying the induction of *vtg* across laboratories is a challenge from both a technical and biological variability standpoint. Moving forward, quantifying estrogen-responsive gene networks may improve both accuracy and sensitivity when monitoring effluent quality over site, time, and across laboratories in small bodied fish. The objective of this study was to identify core transcriptional networks in the liver that are responsive to estrogens by leveraging data across six different laboratories as well as resources from the Comparative Toxicogenomics Database.

2. Materials and methods

2.1. Experimental design for inter-genomics study

All experimental procedures described herein were approved by the Animal Care Committee (protocol number 2013-3s-09) and carried out at the Canadian Rivers Institute at the University of New Brunswick, Saint John, NB, Canada. Full details on the experimental design can be found in Feswick et al. (2017). Briefly, male FHMs aged 1.5 years were exposed to 17alpha-ethiylestradiol for 96 h in a static renewal exposure design (water was renewed every 24 h with 100% water change). Measurements of EE2 verified that the mean concentration (± SEM) of EE2 in the tanks was 15.67 ± 4.71 ng/L, and plasma Vtg levels were 4.0 ± 12.7 μg/mL for control males (n = 8) and 21,666 ± 1821 μg/mL for EE2-treated males (n = 8), confirming a treatment effect and biological response to the estrogen (Feswick et al., 2017). There were no significant differences in body weight, body length, gonadosomatic index, or hepatosomatic index between the control and EE2-treated group.

Sixteen liver samples (8 control and 8 treated) were partitioned equally and shipped on dry ice by courier in numbered (blinded) vials to each of six laboratories with experience in microarray analysis. A 60 K probe fathead minnow microarray was used by each laboratory and data are available in NCBI Gene Expression Omnibus (GPL15775 Agilent-036574 FHM_8 × 60K_V2, BioProject PRJNA321209). Gene expression data were returned to the coordinating laboratory at University of New Brunswick, and the methods for microarray analysis were reported (Feswick et al., 2017).

2.2. Inter-laboratory comparison: pathways

Raw intensity data from each laboratory were imported one at a time into JMP® Genomics v 7.0 (SAS Institute Inc., Cary, NC, USA). Intensity data were normalized for each laboratory using quantile normalization. Control probes were filtered out prior to identifying differentially expressed genes (DEGs). Datasets were filtered to the average intensity of the 8th Agilent spike across all spots and arrays [(+)E1A_r60_a107 (spike 8)], the rationale being that, upon viewing all quality control reports, the 8th spike was consistently the last point of a linear standard curve. The limits of detection for intensity are provided in Feswick et al. (2017). DEGs were identified using a one-way analysis of variance (ANOVA) followed by a false discovery rate (FDR) set at 5.0%.

Data from each of the six laboratories were analyzed individually for pathways. Pathway Studio 9.0 (Elsevier) and ResNet 10.0 were utilized for sub-network enrichment analysis (SNEA) of cell processes (Nikitin et al., 2003). The option of "Highest magnitude fold change, best p value" in Pathway Studio was used for duplicated probes. A total number of 37,169 fathead minnow probes were successfully mapped to the program using the official gene name (Name + Alias). SNEA was conducted to identify gene networks that were affected in the FHM liver following EE2 exposure. Networks were constructed based upon common regulators of cell processes. The enrichment P-value for a gene seed was set at $P < 0.05$. Additional details on the use of SNEA can be found elsewhere (Langlois and Martyniuk, 2013).

2.3. Transcriptional targets of 17β-estradiol: the comparative toxicogenomics database

To compare our liver networks to that which is known in the literature, we extracted all the transcripts that have been reported to be regulated by 17beta-estradiol (MeSH® ID, D004958) from the Comparative Toxicogenomics Database (CTD). The CTD is a publicly

Download English Version:

https://daneshyari.com/en/article/8546106

Download Persian Version:

https://daneshyari.com/article/8546106

Daneshyari.com