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Genetic and epigenetic determinants of inter-individual variability in responses to toxicants

Lauren Lewis[1], Gregory E. Crawford[2], Terrence S. Furey[3] and Ivan Rusyn[1]

Abstract

It is well established that genetic variability has a major impact on susceptibility to common diseases, responses to drugs and toxicants, and influences disease-related outcomes. The appreciation that epigenetic marks also vary across the population is growing with more data becoming available from studies in humans and model organisms. In addition, the links between genetic variability, toxicity outcomes and epigenetics are being actively explored. Recent studies demonstrate that gene-by-environment interactions involve both chromatin states and transcriptional regulation, and that epigenetics provides important mechanistic clues to connect expression-related quantitative trait loci (QTL) and disease outcomes. However, studies of Gene × Environment × Epigenetics further extend the complexity of the experimental designs and create a challenge for selecting the most informative epigenetic readouts that can be feasibly performed to interrogate multiple individuals, exposures, tissue types and toxicity phenotypes. We propose that among the many possible epigenetic experimental methodologies, assessment of chromatin accessibility coupled with total RNA levels provides a cost-effective and comprehensive option to sufficiently characterize the complexity of epigenetic and regulatory activity in the context of understanding the inter-individual variability in responses to toxicants.

Addresses

[1] Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA

[2] Center for Genomic and Computational Biology and Department of Pediatrics, Division of Medical Genetics, Duke University, Durham, NC, USA

[3] Department of Genetics, Department of Biology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Corresponding author: Rusyn, Ivan (irusyn@cvm.tamu.edu)

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1. Genetic variability

Estimation of the degree of inter-individual variability in the population is a required step in assessing the human health hazard posed by environmental chemicals. Indeed, the National Academies report *Science and Decisions* [1] called for the need to better "*account for differences among humans in cancer susceptibility other than from possible early-life susceptibility.*" Recent advances in the ability to conduct genome-wide association studies (GWAS) that identify quantitative trait loci (QTL) have enabled identification of genetic variants associated with important diseases [2]. It is clear that genetic variation influences the response of an individual to drugs and chemicals [3]. The blossoming field of personalized medicine now brings GWAS-enabled understanding of basic biology into clinical practice to determine how the knowledge of genetic variation can make therapies safer and more effective by tailoring selection and dosing of drugs for an individual patient [4].

GWAS that characterize effects of environmental toxicants on humans are usually based on epidemiological data, not controlled exposures [5]. This makes it a challenge to interpret findings from human cohorts exposed in the occupational or environmental settings. In addition, collection of tissues (with the exception of blood) from a wide variety of anatomical sites or developmental stages is not possible in humans that have been exposed to environmental toxicants. These limitations can be alleviated, at least partially, by the use of appropriate genetically-diverse laboratory animal-based model systems [6].

The mouse is a popular *in vivo* model for which genetic resources with publicly available genetic maps across dozens of strains are now available [7]. Mouse populations, such as the Collaborative Cross [8], provide an excellent testing system for evaluation of complexities in toxicokinetics and toxicodynamics [6,9–11]. In the past decade, it has been demonstrated convincingly that genetic diversity in the mouse can be used to identify sensitive sub-populations using a mouse model of the human population approach [12–25]. Most of the genetic variability among mouse strains has been focused on SNPs; however, variation in structure of DNA regions affecting DNA sequence length and/or orientation that includes deletions, insertions, copy-number gains,

inversions, and transposable elements, may also underpin susceptibility traits [26]. In addition, while inbred mouse strains are considered isogenic, intra-strain differences and their influence on experimental outcomes have been identified [27,28].

While advances in sequencing technologies, statistical genetics analysis methods and clinical trial designs have shown promise for the discovery of variants associated with drug response, interpretation of both human and mouse GWAS through identifying causal variants is a challenge, and the translation of the findings to the clinic and/or regulatory actions is slow. On the one hand, it remains difficult to interpret the outcomes of GWAS and validate genes underlying QTLs with certainty, due in part to not knowing which organs, tissues, and/or cell types any particular QTL is having a significant functional effect. On the other hand, the GWAS-driven attempts to disentangle treatment responders from non-responders via genetic predictors in pharmacogenetics studies have not been uniformly successful [29].

2. Linkages between genetic, transcriptional, and epigenetic variability

Comprehensive maps of human and mouse regulatory DNA were recently published by the ENCODE (Encyclopedia of DNA Elements) Consortium [30], mouse ENCODE [31], and the Roadmap Epigenomics Project [32]. These studies comprehensively characterized the location and relationships between chromatin accessibility, histone modifications, chromatin looping, transcription, DNA methylation and the occupancy of sequence-specific factors. The wide spectrum of different cultured cell lines and tissues that were assayed have identified over a million common and cell-type specific gene regulatory elements. Genome-wide chromatin accessibility analyses, originally performed by DNase-seq [33] and more recently by ATAC-seq [34], have become invaluable approaches for mapping the genomic location of transcriptionally-active chromatin. While consortia such as ENCODE and Roadmap have identified large numbers of putative regulatory elements, little is known about how these elements are affected by variation in genetics, sex, or exposure to individual or complex combinations of environmental stimuli.

Studies in a large and genetically heterogeneous collection of human lymphoblast cell lines (LCLs) [32] and tissues [35] have identified heritable variation in gene expression across humans. These expression quantitative trait loci (eQTL) studies have been complemented by a more limited number of chromatin studies that have identified QTLs that impact DNaseI sensitivity (dsQTL; [36]), histone modifications in chromatin (cQTLs; [37]), DNA methylation [38], and transcription factors binding sites [37,39]. These studies demonstrate the versatility and complexity of gene regulation, whereby modulation of gene expression is executed by different elements forming intricate networks that include changes in chromatin activity. In addition, these studies show how genetic variants identified in GWAS can be linked through a regulatory network to the associated gene. For example, it was shown that both locally and distally acting genetic variants exhibit strong influence on expression and chromatin [37,40]. It was also found that two-thirds of local eQTLs were also local dsQTLs or cQTLs [36], which means that the variation in chromatin is associated with variation in the expression levels of nearby genes. At the same time, a total of 15% of proximal histone QTLs were associated with changes in chromatin states at distal genomic regions with which they interact physically [41]. These data show that specific genetic variants modulating regulatory element activity may concordantly affect local and distal chromatin modifications and gene expression.

While population variability in DNA- and chromatin-related epigenetic marks is well recognized, it has been shown that variability in miRNA expression in the population may be negligible as compared to the genetically-determined variability in mRNA expression [20,42]. Specifically, few eQTLs were observed for miRNAs in various tissues in population studies in mice [13,43,44]. The stability of miRNA expression in a genetically diverse population suggests that miRNAs may be a much more reliable population-wide biomarker of the effects of chemicals on epigenetic mechanisms of toxicity, as compared to changes in DNA methylation, chromatin and/or histone modifications. Indeed, chemical-induced disruptions in miRNA expression, a phenomenon established for a large number of toxicants, is recognized as an important toxicity mechanism [45]. Post-transcriptional regulation of mRNA levels by miRNAs is not a true epigenetic process. For the remainder of this review, though, we include miRNAs when discussing the epigenome for the sake of simplicity as their primary function is to regulate gene expression.

3. Environmental agents cause toxicity through epigenetic mechanisms

Epigenetic reprogramming has been proposed as an integral part of the "genome instability" enabling characteristic of cancer cells [46] and it is well established that chemical carcinogens may affect the cellular epigenetic state [47]. Changes in DNA methylation, histone/chromatin remodeling, and altered expression of miRNAs represent the most frequently reported toxicant-induced alterations of the epigenome [48]. Because of the potential impact of these epigenotoxic